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The Chemistry of the Injured Cell

Publication Number 452

AMERICAN LECTURE SERIES®

A Monograph in

AMERICAN LECTURES IN LIVING CHEMISTRY

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The Chemistry of the Injured Cell

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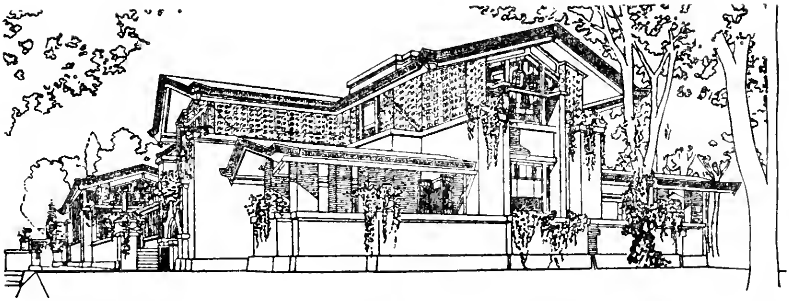

CHARLES C THOMAS · PUBLISHER
Springfield · Illinois · U.S.A.

CHARLES C THOMAS • PUBLISHER
BANNERSTONE HOUSE
301-327 East Lawrence Avenue, Springfield, Illinois, U.S.A.

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Library of Congress Catalog Card Number: 61-9027

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To
R. A. Willis

FOREWORD

Our Living Chemistry Series was conceived by Editor and Publisher to advance the newer knowledge of chemical medicine in the cause of clinical practice. The interdependence of chemistry and medicine is so great that physicians are turning to chemistry, and chemists to medicine in order to understand the underlying basis of life processes in health and disease. Once chemical truths, proofs and convictions become sound foundations for clinical phenomena, key hybrid investigators clarify the bewildering panorama of biochemical progress for application in everyday practice, stimulation of experimental research, and extension of postgraduate instruction. Each of our monographs thus unravels the chemical mechanisms and clinical management of many diseases that have remained relatively static in the minds of medical men for three thousand years. Our new Series is charged with the *nisus élan* of chemical wisdom, supreme in choice of international authors, optimal in standards of chemical scholarship, provocative in imagination for experimental research, comprehensive in discussions of scientific medicine, and authoritative in chemical perceptives of human disorders.

Dr. Cameron and Dr. Spector of London reveal the chemical spectrum of body reactivity to environmental injury, intrinsic and extrinsic, in its effort to preserve the integrity of the whole organism and of its constituent parts. The chemical approach is a medical milestone in the march of the human mind. Pathology initiated by Morgagni from the structural standpoint in organs, has been studied successively by Bichat in tissues; by Virchow in cells; and now by Cameron and others from the functional viewpoint in molecules and submolecules. The sequence of events which constitutes the living response to pathological injury is gradually being elucidated while different facets of the clinical picture are being obtained through experimental exploration of cell chemistry by the newer techniques. It remains to be determined whether the response is the resultant of a chain reaction or a collateral series of reactions. John Hunter in 1794 thought that these changes dispose towards recovery but the authors consider them mixed blessings. The clinical interpretation of the biochemical response to injury thus enables us to recognize what was formerly imperceptible in disease processes, whether it be passive submission, active resistance, or effective adaptation to bodily injury.

I. NEWTON KUGELMASS, M.D., PH.D., SC.D., *Editor*

INTRODUCTION

In terms of the body, injury may be defined as a harmful event or an unfavourable environmental development, intrinsic or extrinsic. Pathology is the study of such injury and of the body's reaction to it, and injury and reaction to injury combine inextricably to produce the state we recognise as disease.

Pathology was first studied in a purely structural frame of reference first in organs, then in tissues, finally in the cell. Hard on the heels of morbid anatomy came the recognition of disordered function, to be followed by the exploration of abnormal cell chemistry.

It is this latter phase of the study of disease with which this small book is concerned and it will be quickly apparent to the reader that the chemistry of cell injury is still in the infantile stage of development. Indeed, there are great sectors in which virtually nothing is known and we have not deemed it desirable to attempt to conceal these deficiencies. On the other hand where sufficient reliable evidence has been accumulated to justify formulation of an explanatory hypothesis this has been presented, albeit with the reservation that further evidence may destroy what others have devised.

Finally, certain topics have been omitted as an act of policy. Examples of this exclusion are carcinogenesis and virus-host interrelationships. These aspects of cell injury constitute major subjects in themselves and have been amply discussed elsewhere by specialists. Even in the spheres which have been discussed it will be apparent that there has been no attempt at complete coverage, but rather an effort to use examples of natural and experimental disease as illustrations of a general theme.

The authors would like to express their indebtedness to their colleagues, Drs. M. J. R. Dawkins and K. R. Rees for much invaluable discussion and to Mr. V. K. Asta for preparing the figures. A special acknowledgement is due to Dr. A. E. McLean for his co-operation in the preparation of the chapter on electrolytes. Some of the data and discussion attributable to Dr. McLean form part of his thesis for the degree of Ph.D. in the University of London.

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The Chemistry of the Injured Cell

PART I

THE CHEMISTRY OF CELL INJURY IN RELATION TO PARTICULAR CELLULAR CONSTITUENTS

Chapter 1

WATER AND ELECTROLYTES

IT IS WELL KNOWN that when mammalian cells and tissue slices are exposed to an unfavourable environment in an isotonic medium in vitro the cells suffer an increase in volume and in weight. It is also known that the increase in weight is due to accumulation of water within the cell. Equally commonplace is the swelling of cells in vivo demonstrable by standard histological methods which also disclose vacuolation and haziness of the cytoplasm of the swollen cells. These in vivo phenomena, seen in sections of fixed tissues, notably liver, are known as cloudy swelling or hydropic change and represent an early and often reversible indication of cellular injury due to a large variety of damaging stimuli. Mitochondria isolated from cells in this condition have been found, like the cells themselves, to be swollen and laden with water. It has been suggested that some of the vacuolation seen in the cell cytoplasm in cloudy swelling in fact represents such swollen mitochondria.

It is established then that an early sign of cellular damage is an influx of water into the cell. This is almost invariably accompanied by another change, namely an outflow of potassium from cells. The concentration of this ion within the cell is of course normally many times greater than that in the extracellular fluid. Isolated mitochondria, too, lose potassium when they suffer damage.

These phenomena, particularly the influx of water, have been the starting point of many investigations into the water and electrolyte metabolism of cells. It is known from isotope studies that the normal cell is freely permeable to water so the flow of water into injured cells cannot be due to increased permeability to water. Sabbatani (1901) showed that the swelling and water uptake of tissues incubated in vitro in isotonic saline could be prevented by rendering the surrounding medium hypertonic, a finding confirmed on innumerable occasions since. This observation led to the view that the intracellular compartment is hypertonic relative to the extracellular fluid.

The mechanism whereby water is excluded from the normal cell appeared to be revealed by a number of investigations all showing that the exclusion of water appeared to depend on energy producing reactions in the cell. Thus many of the various types of injury leading to swelling of tissues in vitro interfere with cell metabolism. Anaerobiosis, low temperature, poisoning with 2:4 dinitrophenol, cyanide, azide or heavy metals, all block the oxidative formation of high energy phosphate bonds and all cause water influx into cells in vitro. In red blood corpuscles, too, the general principle holds.

Robinson (1950) showed that on incubation in hypotonic surroundings, metabolising tissue slices became water-logged less rapidly than did similar slices whose metabolism had been inhibited by cooling to 0°c. His experiments seemed to support the argument that water was prevented from entering a normally hypertonic intracellular compartment by energy-yielding metabolic processes.

Two sets of observations have served to invalidate this view. The first observations concern direct measurement of the intracellular tonicity. This was first attempted by determination of the freezing point of tissues as compared with that of blood. All such experiments have shown tissues to yield values about 150 per cent of those of blood. A similar result was obtained by measurement of melting point depression. The effect of these findings was apparently to confirm the view that the cell was hypertonic relative to its surroundings.

However, this interpretation was challenged because it was shown that after removal from the body, even at 0°, there occurred in the tissues a very rapid rise in the value for freezing point depression due presumably to autolysis (Conway and McCormack, 1953). In fact, a calculation was made showing that if corrected for post mortem changes, the freezing point depression of tissues was similar to that of blood. These conclusions did not pass unchallenged but freezing point determinations on tissues previously heated to prevent autolysis indicated that the cells were in fact isotonic with blood (Appleboom, 1957). The truth of this statement is indicated by recent experiments quoted by Leaf (1959). In these, melting point depression was utilised and autolysis and thermal artefacts excluded by the use of very low temperatures. The results show without doubt that the intracellular compartment is isotonic with the extracellular fluid.

The second blow to the view that failure of water extrusion was the basic cause of swelling of injured cells came from direct measurement of other ionic concentrations after injury. Thus slices of kidney or other tissues injured by chilling, oxygen lack or metabolic poisons show a net uptake not only of water, but also of sodium and chloride (Mudge, 1951; Leaf, 1956). The inflow of chloride ion into the injured cell is particularly striking and indicates clearly that in injury there is an entry of isotonic extracellular fluid into the cell and that the defect is not essentially a disorder of water transport.

In summary, in tissue injury there is an influx of isotonic fluid into an isotonic cell. Ions other than sodium and chloride may enter, notably calcium which may accumulate within the cell to a considerable extent. These events are of course associated with a massive loss of potassium from the cell into the environment.

Relative to the extracellular fluid the cell is rich in potassium and lacking in sodium and chloride. However, the cell wall is freely permeable to all these ions in the sense that intracellular sodium, potassium and chloride will exchange readily with similar extracellular ions, the movements being followed with the aid of isotopes. If a normal cell is placed in a solution of potassium chloride, the potassium ion enters the cell and accumulates therein until the cell swells and possibly dies. A similar experiment in sodium chloride, however, is not followed by intracellular accumulation of sodium. Thus in another sense the permeability of the cell to sodium is restricted.

Much evidence indicates that these ionic gradients are maintained by the expenditure of energy derived from cell metabolism. The detailed picture of this mechanism and of its failure after injury remains obscure. It is, however, a problem which lies at the heart of contemporary physiology and pathology.

Any hypothesis advanced must take into account six basic facts. These are:

1. The existence of sodium exclusion and of potassium accumula-

tion by the normal cell, i.e. the maintenance of ionic gradients.
2. Free permeability of the normal cell ~~wall~~ membrane to water, sodium, potassium and chloride in the sense indicated above.
3. The existence of a rapid sodium and potassium exchange between the cell and the extracellular fluid not dependent upon the expenditure of energy.
4. The existence of intracellular isotonicity.
5. The existence of an electrical potential across the normal cell ~~wall~~. membrane
6. The loss of ionic gradients by the injured cell and their restoration with recovery when injury is reversible.

The classical and still widely accepted explanation is based on the concept of a "Sodium Pump." The theory suggests that intracellular sodium exchanges freely with extracellular sodium on a one-for-one basis but that there exists in addition a "leak" of sodium ion into the cell from the higher concentration outside the cell wall. The hypothesis postulates an active extrusion of sodium (the Sodium Pump) sufficient to counterbalance this leak and dependent upon the expenditure of energy. As a result of this extrusion of sodium there is a potential excess of intracellular anions. It is postulated that potassium is the only cation available to neutralise this anionic excess and that the existence of a concentration gradient for potassium is therefore part of Donnan equilibrium and a result of the active extrusion of sodium. The "Sodium Pump" is thought also to account for the existence of an electrical potential across the cell wall. It is held that the exclusion of chloride ion from the cell is again a consequence of the electrical potential caused by the extrusion of sodium, the cell interior being relatively electronegative.

It is convenient at this point to consider what picture emerges of the normal and injured cell when the interpretations summarised above are accepted. Putting the concept in its simplest possible terms, the cell is seen as a sac bounded by a semipermeable membrane. Within the cell electrolyte ions are in a freely diffusible state and there is a rapid and continuous exchange between the intracellular ions and their extracellular counterpart. The intracellular colloids exert oncotic pressure which would lead to intracellular accumulation of water and consequent cellular swelling were it not for the active extrusion of sodium.

When the cell is injured, energy may no longer be available for the extrusion of sodium. As a result, the extracellular sodium which continues to "leak" into the cell accumulates therein. These sodium ions neutralise anions e.g. in protein molecules, thus disturbing the Donnan equilibrium and allowing potassium to leave the cell. In addition, chloride ion enters the cell partly because of loss of polarity by the cell membrane. As a result, more sodium enters to maintain electrical neutrality. Thus the entry of sodium and chloride exceeds the loss of potassium, hence there is a net uptake of water by the cell which consequently undergoes swelling.

The classical view of cellular water and electrolyte metabolism and the derived explanation of the water and electrolyte movements seen in cell injury appear satisfactory. Unfortunately, rigorous experiments, especially those measuring isotope exchange, have indicated that in many cases the observed facts are not explicable on the basis of a "sodium pump" alone. As a result, it has proved necessary to postulate the existence of separate energy-dependent transport mechanisms for potassium, chloride and water, in addition to the "sodium pump" (Leaf, 1959; Rothstein, 1959). The proposed mechanisms for these multiple transport systems include contractile and re-orientating proteins and a variety of carrier systems, diffusible and fixed.

The point of such modifications of the classical hypothesis is to fit a view of the cell as a water-filled sac containing electrolyte ions in a freely diffusible state. In recent years this picture has been challenged by an alternative concept.

The classical view of the cell considered that such barrier as existed between ions e.g. K ions of cell and environment was merely a thin membrane, the cell wall, and that within the cell the ions moved freely. Actual determinations of the mixing of isotopically labelled K ions revealed, however, that the situation was more complex and that mixing did not proceed in as uncomplicated fashion as it should were this concept of the cell a valid one. To explain these discrepancies it was suggested that intracellular potassium ions existed in two compartments, one exchangeable with extracellular potassium, the other not exchangeable. However this theory is unattractive and the failure of isotopic K to mix uniformly with intracellular K could be explained better by the view that the diffusion of K inside the cell is not in fact free but is on the contrary restricted. In other words, the movement of ions between intra- and extra-cellular fluids is governed not only by the characteristics of the cell membrane but also by those of the cytoplasm.

Harris (1957) has shown that if the cell resembled a model in which a restrictive membrane enclosed a space in which potassium ions moved freely, then for a cell of 80 μ diameter equilibration of intra- and extra-cellular potassium should be 90 per cent complete in less than one second. In fact, mixing occurs very much more slowly. By studying in muscle the uptake of tracer potassium Harris has shown also that the intracellular potassium, although diffusing slowly, possesses uniform diffusion properties.

These results, and others, indicate that intracellular potassium is not in separate compartments but is in a uniform state. The most popular conception of this state is that the potassium ions are held by absorption on anionic sites, e.g. proteins, nucleic acids, or phospholipids. Potassium exchange would then depend on the displacement from its locus of one ion by another and would therefore be slow enough to account for the experimental findings in isotopic mixing.

As far as potassium is concerned, the cell may then be considered as a lattice work or matrix on which are absorbed the potassium ions, while in the interstices of the network move the ions which have been displaced or which have yet to occupy a site. It may be that the endoplasmic reticulum is the structural basis of this ion-binding matrix.

It seems likely that the movement of sodium ions, too, into and out of the cell is governed not only by membrane permeability but also by limited diffusion within the cell (Harris, 1957). This suggests that part of the matrix is occupied by sodium. Intracellular sodium and potassium tend to vary inversely so that if the latter concentration is raised due, for example, to an environment rich in potassium, intracellular sodium tends to fall. This could be explained on the assumption that there is a limited degree of cross specificity with regard to sites binding both ions. For a variety of reasons, however, it is generally preferred to postulate separate sodium-rich and potassium-rich zones within the cell. In particular, the facts suggest at present that the sodium-rich zone is at the periphery of the cell (in the sense of being more accessible to isotopic exchange) and consists perhaps of an annulus about 3 μ thick.

Thus this current concept of the cell postulates an ion-binding matrix, part specific for potassium, part accessible to other ions including sodium, the sodium zone at the "periphery," the potassium at the "centre," the potassium binding sites being inaccessible to sodium provided that the cell retains its integrity. It is also likely that although potassium is present in the interstices, the binding sites in the sodium zone are inaccessible to potassium ion in the intact cell.

The substitution of ion exchange within the cell for the classical view of electrolyte metabolism is compatible with the facts presented above. The theory is also compatible with observations concerning electrolyte movements in injury. It is necessary to assume that the specific ion-binding properties of the matrix, particularly for potassium are dependent on the integrity of this structure which in turn depends ultimately but not exclusively upon metabolic reactions and that in the early stages of injury, such a loss of specificity is reversible. Thus the exchange of extracellular potassium with potassium loosely bound to the matrix would not of itself require energy; the exclusion of ions other than potassium from the ion-binding sites might require active metabolism, if only to maintain the structural integrity of the ion-binding system.

There is good evidence that discrepancies may exist between the metabolic integrity of cells and their ability to maintain normal electrolyte gradients. Thus strophanthin and tetrabutylammonium affect ionic movements without apparently having the expected adverse actions on cell metabolism (acetyl choline and adrenaline have similar properties). Harris has suggested that in injury of the strophanthin type there is not so much loss of potassium-binding specificity as expansion of the sodium-rich at the expense of the potassium-rich zone. As a result, potassium uptake would be slowed due to fewer potassium-binding sites and to increased difficulty in reaching the inner potassium-rich zone. Simultaneously, more of the sodium ions normally entering the cell will remain in the cell because of more sodium-binding sites. Thus sodium would displace potassium in the intracellular fluid. These changes would explain the observed facts that in cells so injured there is reduced output of sodium, increased output of potassium and that the net loss of potassium equals the net gain of sodium.

It is clear that there are two rival views of the mechanism whereby cells maintain electrolyte gradients. One view takes the membrane to be the major factor and the internal electrolytes to be freely diffusible. The opposing theory believes specific ion-binding within the cell to be of greater importance. It is obvious that any explanation of electrolyte movements in cell injury must be based on one or the other of these hypotheses.

The ion-binding theory has been criticised by Rothstein (1959) chiefly on the grounds that no evidence is available of specific potassium or sodium binding by cell constituents. However, since it is now necessary for the protagonists of the membrane theory to postulate specific transport systems or "pumps" for ions other than sodium, a similar objection applies to the membrane hypothesis.

A dispassionate study of the recent literature reveals that there is much to be said for and against both theories. Electron microscopy is now revealing that the cell membrane is not merely a boundary structure but is on the contrary in intimate and variable contact with all parts of the cell interior, as well as being the seat of many metabolic reactions and containing ion-binding radicles. This new concept of the membrane as both cell boundary and an actively metabolising part of the cell interior may perhaps serve to bridge the gap between the two hypotheses of electrolyte transport.

The relation between in vitro injury such as chilling of tissue slices and injury *in vivo* may be indicated by some recent experiments carried out at University College Hospital by McLean. Young rats fed on the deficient diet described by Himsworth develop hepatic necrosis, which appears rather suddenly at about seventeen days. McLean prepared slices from the livers of these animals a few days before necrosis appeared and compared their behaviour with normal liver slices. He found that as expected both types of liver slice lost potassium and accumulated sodium and water when placed in cold saline. When transferred to oxygenated Ringer solution at 37°, the normal liver slice reaccumulated potassium against a concentration gradient. However, the pre-necrotic liver slices from rats fed a deficient diet failed to do so as early as three days after commencing the diet. Inability to reaccumulate potassium precedes any demonstrable decline in oxygen uptake by at least ten minutes. The addition of phenergan (promethazine HCl, a compound active in permeability phenomena) to the medium restores the ability of pre-necrotic liver slices to reaccumulate potassium and to extrude sodium and water and prevents the decline in oxygen uptake. The potassium content of livers from dietetically deficient rats is not obviously lowered until necrosis supervenes. Thus these results suggest that the earliest changes in one type of liver injury, namely dietary deficiency, are a failure to maintain electrolyte gradients in adverse circumstances, possibly followed by a similar failure in normal circumstances. The results suggest also that as in the strophanthin experiments, disturbances in water and electrolyte balance may in fact precede abnormalities in energy-yielding metabolic activity and may even precipitate such abnormalities.

Other results obtained by McLean show that liver cells from rats deficient in vitamin E exhibit a particular readiness to lose potassium and to gain sodium in vitro. This behaviour, too, is reversed by the addition of phenergan and related compounds, all of which are anti-oxidants, as is vitamin E itself. These experiments suggest a possible mechanism, other than general metabolic disturbance, for the characteristic changes in electrolyte movement following injury. It may be that specific ion-binding groupings on molecules such as phospholipids are especially susceptible to auto-oxidation with or without the intervention of free radicles such as Fe^{++}. Vitamin E might act as a natural anti-oxidant to prevent this reaction and phenergan and chlorpromazine might exert a similar effect, although there are alternative explanations for the action of these phenothiazines (see below) . It would have to be assumed that auto-oxidation would destroy the specificity of the ion-binding groups but such groups could form part of both a membrane transport system and an internal ion-binding matrix. In view of the protective effect of vitamin E and phenergan in dietary liver necrosis and of phenergan in hepatic necrosis induced by carbon tetrachloride and thioacetamide (Rees, Spector and Sinha, 1961), it is possible to postulate auto-oxidation as a potential pathological mechanism in conditions other than vitamin E deficiency. Conway (1955) has suggested that ion transport may depend upon oxidation-reduction reactions in the cell membrane, with electron transport through iron-containing enzymes. The theory postulates that when the complex is reduced, there is specific absorption of sodium or potassium ions on to the negative charge thus produced. With oxidation, the ion is released. Conway's theory has been challenged but it is easy to see that his hypothesis is consistent with that based on the actions of anti-oxidants in cell injury associated with vitamin E deficiency. Although acute disturbances may occur in the presence of normal metabolism it is abundantly clear that sustained maintenance of normal ionic gradients depends directly or indirectly on energy transformations within the cell. This is true whether the important mechanisms are ionic pumps or ion-binding matrices. It is also known that ionic gradients do not necessarily depend on ATP, the major source of stored energy, itself. Some recent work of Judah (1960) indicates that at least one of the energy transforming reactions closely concerned with electrolyte metabolism may be the phosphorylation and dephosphorylation of cellular phosphoproteins. Thus certain phenothiazines, notably phenergan (promethazine HCl) inhibit the incorporation of phosphate into phosphoprotein in parallel with similar inhibition of electrolyte movement. Should further work confirm the importance of the phosphoprotein system and the impression of its nature now obtaining, it would seem that its characteristics favour an ion-binding matrix (perhaps the phosphoproteins itself) rather than an ionic pump.

CALCIUM AND CELLULAR INJURY

The Deposition of Calcium in Damaged Tissues (Dystrophic Calcification)

Certain types of tissue damage are commonly associated with the deposition of calcium salts in the affected region. Notable examples are tuberculous lesions showing coagulation necrosis (caseation), necrosis in fat, arteriosclerosis and infarcts (necrosis due to sudden loss of blood supply). The nature of these calcium deposits appears to be similar to that found in normal bone, i.e., to consist of hydroxy apatite ($Ca_{10}[Po_4]_6(OH)_2$) on which is absorbed carbonate, magnesium and sodium ions.

The mechanism of both normal and dystrophic calcification is very uncertain. In normal calcification several factors have been implicated; a supply of glycogen, the formation of a phosphate ester by the process of glycolysis, the breakdown of this ester by the enzyme alkaline phosphatase with resultant formation of insoluble calcium phosphate, a reaction between the organic matrix (e.g. sulphate and glucuronic acid groups of chondroitin sulphate) of the tissues and calcium ion, with subsequent calcium binding.

With regard to dystrophic calcification, the early suggestion that fatty acids are formed in necrotic tissue and that these form insoluble calcium soaps later replaced by calcium phosphate, is no longer held to be valid. Increased alkalinity of necrotic tissue as a cause of precipitation of acid-soluble calcium salts has also foundered through lack of evidence. Some sites of abnormal calcification show strong phosphatase activity, e.g. caseous tubercles in rabbits, phosphatase not appearing until necrosis was apparent. This observation suggests that phosphatase activity may be involved in both normal and dystrophic calcification. The enzyme might well be liberated from intracellular sites, e.g. mitochondria after cell death. However, not all types of calcifying degenerate tissue show phosphatase activity. This type of abnormal calcification may be favoured by the development in degenerate tissues of greatly increased calcium binding properties or by the loss of substances that normally serve to keep calcium in a non-ionic but soluble form.

Metastatic Calcification

In this condition, essentially normal tissues, particularly kidney tubules, lung alveoli, thyroid and gastric mucosa, become the site of calcium phosphate precipitation. The lesions are due to abnormal concentrations of calcium and phosphate in the blood.

The cause of the abnormal blood levels may be over-activity of the parathyroid gland or more rarely hypoparathyroidism, excessive doses of vitamin D or destructive lesions of bone, e.g. secondary carcinoma. Other causes also exist. The essential feature of this type of calcification is loss of calcium from the bones and its deposition elsewhere. Much recent work has been performed on this subject and it will not be pursued further here.

Intracellular Accumulation of Calcium

This phenomenon is known to occur as part of the disturbance of electrolyte movements seen in cells and tissue slices in an unfavourable environment *in vitro*. In addition, at least two types of liver damage, due to carbon tetrachloride and thioacetamide show in vivo intracellular calcium accumulation. This feature of cell injury appears to be related to abnormal cell permeability, the calcium possibly entering to maintain electrical neutrality in the face of potassium loss, although the situation may be in fact more complex. The massive entry of calcium may be particularly important in view of the toxic effect of high concentrations of this ion on respiratory enzyme systems. The calcium influx may thus be both a result and a further cause of cellular injury.

REFERENCES

Appleboom, J. W. (1957) : *Fed. Proc., 16:*278.
Conway, E. J. (1955) : *Intern. Rev. Cytol., 4:*377.
Conway, E. J. and McCormack, J. I. (1953) : *J. Physiol., 120:*1.
Harris, E. J. (1957) : *J. gen. Physiol., 41:*169.
Judah, J. D. (1960) : *Exp. Cell Res., 19:*404.
Leaf, A. (1956) : *Biochem. J., 62:*241.
Leaf, A. (1959) : *Ann. N. Y. Acad. Sci., 72:*396.
McLean, A. (1960) : *Nature, 185:*936.

Mudge, G. H. (1951) : *Amer. J. Physiol., 167*:206.

Rees, K. R., Spector, W. G. and Sinha, K. P. (1961) *J. Path. Bact., 81*: 107.

Robinson, J. R. (1950) : *Proc. Roy. Soc.,* B., *137*:378.

Rothstein, A. (1959) : *Bacteriol. Rev., 23*:175.

Sabbatani, L. (1901) : *J. Physiol. et pathol. gen., 3*:939.

Chapter 2

FATS

WE MUST NOW SEE WHAT happens to the fats in a damaged cell. This means that we must consider, in the first place, the conditions that upset the metabolism of fat in a broad sort of way, then we must try to find out how much and what kind of fat accumulates under such circumstances and finally, we shall select recent discoveries that help us to explain the meaning of these changes.

WHAT MAKES THE CELL "FATTY"?

For a long time we have known that certain organs become very fatty as the result of disease. The liver, kidneys and heart often are affected in this way, and experience has shown that whenever this happens, the individual has been exposed to severe infection, intoxication with one or other of a large variety of poisons, a restricted oxygen supply or possibly inanition. Sometimes the patient is overnourished, in which case his connective tissues are overloaded with fat and it would almost seem, too, as if the organs were participating in a generalised adiposity.

How to assemble such a wide array of causes into a few generalisations has vexed investigators for many years until the ingenious experiments of Lebedev (1883) and Rosenfeld (1902, 1903) and the modern versions of Dible, Best and their collaborators effectively sorted out the difficulties. The details of this era of research are given by Cameron (1952). All that we need do now is to remind the reader that the fat depots were "labelled" by feeding animals with an easily-detectable foreign fat and the organs were then damaged by a poison such as phosphorus. As a result the cells of the affected organ became stuffed with fat and when this was analysed it turned out to be chiefly foreign fat. Without doubt this fat has been brought in the blood from the depots to the organs whose cells can no longer use it up or prepare it for transport to other localities because they have been poisoned.

There is, however, another explanation that deserves attention. This arose out of claims that the amount of fat that can be extracted from fatty organs, especially the kidneys, is about the same as that in the normal organ, even though the microscope suggests otherwise. Wells (1925), for instance, found a mere 16 per cent fat in a kidney that looked extremely fatty to the naked eye; the fat content of the normal organ is about 18 per cent. The only way out of this dilemma is to suggest that fat may be liberated or "unmasked" in damaged cells and so lose its close union with other components. These, quite likely, are proteins. Much of the "masked" fat resists calls made on it during starvation, and it cannot be extracted by solvents such as ether. Quite a large part of this lipid is lecithin, cholesterol and their esters. Deliberate digestion by enzymes is needed if the invisible fat is to be set free so as to show up as "fatty degeneration."

But this plausible theory has received a series of hard knocks in recent years from the quantitative studies of Dible and his coworkers and now most of us prefer the infiltration theory as the explanation of fatty change. However, a fresh impetus has come from a totally unexpected direction, for our ideas about the nature of fatty change were largely revolutionised when Banting and Best discovered insulin in 1922. Soon afterwards it was noticed that a dog deprived of its pancreas but kept alive with insulin develops a fatty liver which in time can prove fatal. Feeding the animal raw pancreas prevents this mishap. Subsequent investigation showed that lecithin, and especially choline, which is an important part of the lecithin molecule, is the effective lipotropic agent (Best and Huntsman, 1932). The Canadian workers also discovered that the liver accumulates fat when rats are fed mixed grains and fat. This, too, can be prevented by adding lecithin or choline to the diet. Such studies culminated in the idea of the lipotropic action of protein, cystine and methionine and led to the suggestion by du Vigneaud of transmethylation or transfer of methyl groups. By labelling compounds with isotopes he showed that methyl groups interchange between methionine, creatine and choline, when either one or other is deficient, in an endeavour to synthesise the missing substance. Many donors of labile methyl groups have since been recognised that are directly concerned in the reactions by which phospholipids, especially lecithins, are built up.

When young male rats are fed a low choline diet the fat in their livers increases markedly within forty-eight hours and reaches a maximum after four to six days. Lipid droplets are seen in the liver cells surrounding the central veins within 24 hours and grow into large cytoplasmic masses during the first week of feeding. At this time haemorrhagic degeneration appears in the kidneys along with involution of the thymus and ocular haemorrhage. Liver fat may be increased ten times, but it disappears rapidly when choline is restored to the diet. If the choline deficiency is continued for one to two months, serious liver injury sets in. Cells may rupture and large fatty masses form in the lobules. Eventually the organ becomes cirrhotic, i.e. fibrosed.

Though choline is an important factor in the turnover of lipids and by its absence can induce fatty change in cells, we must not forget that many other causes of this serious disturbance are known to exist. These cannot be prevented from exerting their action by giving choline (Best *et al.*, 1953). However, many animal species respond to choline restoration, including dog, pig and calf, but so far no-one has given direct evidence of such an action in man. It has been argued, largely from experiments with rats, that human alcoholic cirrhosis is the same as the cirrhosis of choline-deficient animals, albeit there are considerable differences in the microscopical picture. We badly need quantitative data on the composition of organs in men exposed to abnormal diets before we can confidently apply the animal results to human beings.

The metabolic aspect of fat disturbance needs further comment. The fat content of cells is, of course, largely influenced by physiological variation in the animal's diet, not only in fat but in carbohydrate and protein. No less important are the various hormones and vitamins that occupy their apportioned places in the series of metabolic changes that determine the fate of lipids after absorption from the intestine until their final utilisation in the tissues. More than a suspicion exists, at any rate in laboratory animals, of the role of vitamins of the B and perhaps E group, hormones from the anterior pituitary gland, adrenals, pancreas, sex glands (oestrogens) in this ordered cycle. Running through the whole set of complexities is, in all likelihood, a number of genetical and constitutional factors which provide the fine adjustment for the more cumbersome homeostatic mechanisms. Each and every one of these guides and catalysts have their work to do in the cell but we are profoundly ignorant of the way in which they achieve their purpose.

HOW MUCH AND WHAT KIND OF FAT ACCUMULATES WHEN A CELL IS DAMAGED?

Information about this key question is sadly lacking, largely because analytical methods of the past were cumbersome and time-consuming. Most of our knowledge is confined to the liver which occupies a central position in the metabolism of lipids. The normal liver contains about 3.5 g. neutral fat, 12-14 g. phospholipid, 0.25 g. total cholesterol (mainly cholesterol esters) per 100 g. fresh tissue. A fatty liver is considerably enriched in its lipid fractions, especially in the neutral fats or glycerides, but the figures vary according to the state of the cells, especially the water content and the extent of associated necrosis. Thus the liver of rats poisoned with bromobenzene shows a decrease in total lipid, lecithin and cephalin, but an increase of total cholesterol when necrosis is pronounced (Cornatzer and Gallo, 1956). High lipid levels (22 per cent and over) have been recorded in fatty livers, with reduction of glycogen, pyruvic acid and α-keto-glutaric acid (Frunder et al., 1955). The extensive fatty change and necrosis in the liver induced by carbon tetrachloride is associated with phosphatide increase followed by decrease in the later stages (Schulze, 1952). Potassium cyanide gives a considerable reduction in the phosphatides of the rat's liver and kidneys with marked reduction in oxygen uptake in their tissues (Goebel et al., 1952). A fatty liver of unknown origin in an eight-year-old donkey contained 80 per cent dry weight lipids, chiefly fats, but only 0.17 per cent phosphatides and 0.4 per cent unsaponifiable fat. Fatty acid analysis showed myristic acid 0.3 per cent, palmitic acid 36.2 per cent, palmitoleic acid 17.9 per cent, stearic acid 3.7 per cent and oleic acid 41.9 per cent (Bernhard and Scheitlin, 1952). Livers of rats receiving intravenous injections of cerium and other rare earths with low atomic numbers develop severe fatty change after forty-eight hours. Liver fat may then be high as 15 per cent (controls 6.11 ± 0.94 per cent), and is mainly neutral fat esters, with no increase in total cholesterol and phospholipid (Snyder *et al.,* 1959).

Some useful information about the human fatty liver is summarised in the following table condensed from Ralli *et al.,* (1941).

MEAN VALUES OF LIPIDS IN NORMAL, FATTY (ALCOHOLIC) AND CIRRHOTIC LIVERS (WET WEIGHTS)

	Total lipids g. per cent	*Fatty acids g. per cent*	*Total cholesterol mgm. per cent*	*Phospholipids g. per cent*	*Neutral fats g. per cent*
Normal (25 cases)	4.98	3.50	283	2.08	2.26
Alcoholics with fatty livers (25 cases)	12.20	11.80	324	1.72	11.4
Cirrhosis of liver (5 cases)	4.94	3.95	283	1.51	3.7

The range of total lipids in these 25 normal livers was 2.42-8.41 g. per 100 g. wet tissue. In the 25 alcoholic cases, 13 had lipid values over 9 g. per 100 g.

The next table gives more information expressed rather differently. It includes some of the figures of Ralli *et al.* re-calculated so as to be comparable with the data of Man *et al.*

RANGE OF LIPIDS IN NORMAL AND DISEASED LIVERS, THE LATTER MOSTLY AFFECTED BY FATTY CHANGE (MAN ET AL)

	Fatty acid m. equiv. per kg.	*Lipid P g./kg.*	*Total cholesterol g./kg.*	*Neutral fat m. equiv. per kg.*
Normal	183-219	1.14-1.28	2.9-3.74	116-150
Ralli et al.'s normals	59-264	0.56-1.17	2.40-3.88	25-191
Liver disease	53-1327	0.7-1.0	2-7	4-1283

Thannhauser and Reinstein (1942) also provide valuable figures for human and fatty livers. The latter were the outcome of subacute bacterial endocarditis, bronchopneumonia, tuberculosis of the lung and alcoholic cirrhosis of the liver. These data show that the outstanding clinical feature in the fatty liver is a pronounced increase in the neutral fat. Phospholipids and especially lecithin are about normal or decreased in amount.

LIPID PARTITION IN HUMAN NORMAL AND FATTY LIVERS
(THANNHAUSER AND REINSTEIN)

	Normal range g./100 g.	*Subacute bacterial endocarditis g./100 g.*	*Broncho-pneumonia g./100 g.*	*Tuberculosis of lungs g./100 g.*	*Alcoholic cirrhosis—range g./100 g.*
Total cholesterol	2.1-2.6	1.98	2.69	2.33	0.88-1.91
Free cholesterol	0.44-0.55	...	0.36	0.27	0.13-0.55
Ester cholesterol	1.50-2.15	...	2.33	2.06	0.72-1.73
Total phospholipids	9-11	7.69	10.6	11.3	3.26-7.34
Sphingomyelin	0.3-0.5	0.19	0.27	0.68	0.04-0.12
Cephalin	3.0-5.5	3.74	...	3.77	0.18-1.94
Lecithin	3.0-6.0	4.76	...	6.85	2.03-4.51
Total fatty acids	8.6-13.0	38.5	15.4	17.3	25.1-66.4
Neutral fat	1.4-4.0	35.1 (approx.)	6.7	8.4	19.8-65.5

Some useful information has come from microchemical and histological analyses of needle puncture material obtained from fatty livers (Billing *et al.*, 1953). 8 non-cirrhotic, non-fatty livers contained 3.5-7.8 g. total lipid per 100 g. wet weight, 5 cirrhotic, non-fatty livers contained 2.3-8.7 g. per 100 g. wet weight. In contrast, 8 non-cirrhotic, fatty livers gave the following figures for total lipid, 8.9-33.5 g. per 100 g. wet weight and 4 cirrhotic fatty livers 14.8-30.1 g. per 100 g. wet weight. Total fatty acid figures parallelled these, showing that an increased neutral fat content was responsible for the fatty change. Phospholipids showed no significant deviation. Some earlier information about fatty liver bears out these conclusions. The average total fat (g./100g. wet wt.) for two human normal livers was 3.25, and for two fatty livers 5.29. Phospholipid percentages were 54.4 and 36 respectively (Theis, 1929).

Neutral fat steadily accumulates in the myocardium after coronary occlusion and in the damaged renal tubule cells after occlusion of the renal artery or exposure to cyanide. We badly need quantitative information in such cases.

THE SIGNIFICANCE OF FATTY CHANGE

The key to the fatty change in the damaged cell lies in our understanding of the metabolism of fat in the normal cell. Fortunately, progress has been impressive in this field of endeavour and we can now apply knowledge about normal processes to the abnormal cell with some confidence, even though important gaps in our knowledge have still to be filled.

BIOSYNTHESIS OF PHOSPHOLIPIDS

Fats are digested in the small intestine into glycerol and fatty acids and these products are absorbed into the lymphatics and blood vessels of the intestinal wall during which process certain syntheses are initiated and perhaps completed. Glycerol, for instance, is phosphorylated through interaction with adenosine triphosphate (ATP) in the presence of the enzyme glycerokinase to give L-d-glycerophosphate. Certain fatty acids are converted into thio esters with coenzyme A and then combine with glycerophosphate to form phosphatidic acid which is promptly reduced to the diglyceride. Meanwhile, choline absorbed from the alimentary canal is brought to the liver where it is phosphorylated by ATP in the presence of a liver kinase to form choline phosphate. From this compound is formed, through interaction with cytidine triphosphate, itself a product of nucleoprotein breakdown, in the presence of a transferase, cytidine-diphospho-choline. Under normal conditions, a transferase catalyses the union of cytidine-diphospho-choline and diglyceride with the production of lecithin. In this form most of the fat of the normal liver, and quite likely of other, cells is stored until required for metabolic purposes; much is liberated from the cells of the liver for utilisation and storage elsewhere in the body. These mechanisms, which are encountered both in living cells and isolated enzyme systems, are summarised in the scheme of Kennedy (1957) to whom we owe so much of this information.

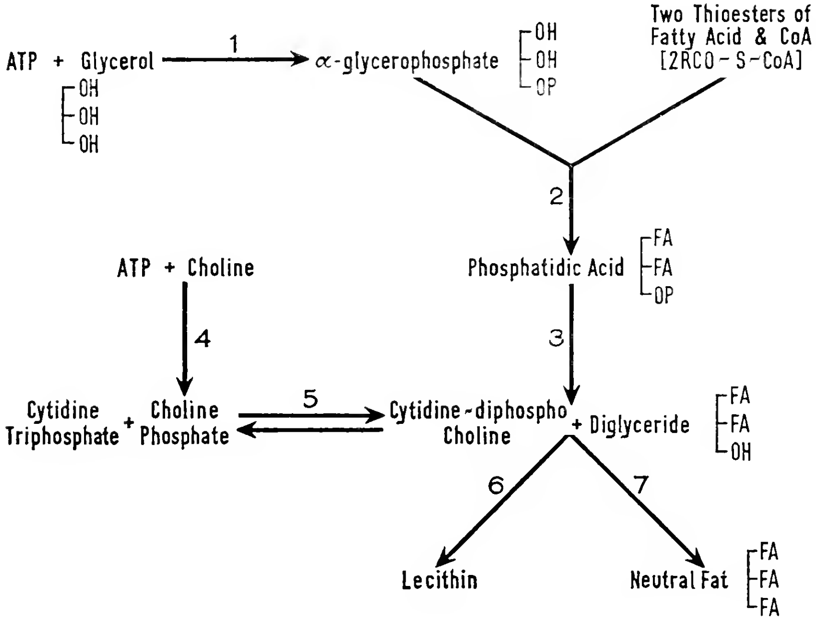

ATP + Glycerol
OH
OH
OH
1
α-glycerophosphate
OH
OH
OP
Two Thioesters of Fatty Acid & CoA [2RCO-S-CoA]
2
Phosphatidic Acid
FA
FA
OP
ATP + Choline
4
3
Cytidine Triphosphate + Choline Phosphate
5
Cytidine-diphospho Choline
+ Diglyceride
FA
FA
OH
6
7
Lecithin
Neutral Fat
FA
FA
FA

Fig. 1. The Biosynthesis of Phospholipid.

For choline there may be substituted ethanolamine or serine as happens during the biosynthesis of phosphatides in the brain and nerves by Schwann cells and probably oligodendroglial cells when they are laying down myelin sheaths around axones.

In addition to these bio-syntheses there are important catabolic processes that make energy available to the cell. Fatty acids, for instance, are broken down to simpler carbon products as part of a process of oxidation which yields CO_2 and water, with liberation of the latent energy of the molecule. This is done through the production of acetyl-Co A units by the process of β oxidation. The 2-carbon fragments are incorporated into the tricarboxylic acid cycle along with similar fragments derived from carbohydrate through pyruvate and oxidised by a series of catalysed reactions to CO_2 and water. Oxidation of fatty acids is exclusively a mitochondrial function. At least part of the oxidation of choline takes place, too, in the mitochondrion. In this way, through a series of remarkable syntheses and reductions, fats are partly converted into phospholipids, partly utilised for energy production with the liberation of water and carbon dioxide.

But there is an alternative reaction at the diglyceride stage. Under certain circumstances we have seen that the cell may be starved of choline; usually this happens because the supply from food or stores in the body is understocked but sometimes it is the outcome of a relative choline deficiency. Our colleagues, Dawkins, Judah and Rees (see below) have shown that this may occur with carbon tetrachloride intoxication. When there is not sufficient choline phosphate for the production of cytidine-diphospho-choline, diglyceride unites with another fraction of fatty acid—Co A and neutral fat or triglyceride is produced. When this happens the cell seems to store the neutral fat as granules or globules which are not made use of for ordinary cellular metabolic activities, and this constitutes the familiar fatty degeneration. This side-tracking of metabolism is the result of a deficiency state or the outcome of a pathological change in the cell. Such is the general theory behind fatty degeneration, not only of liver cells but quite likely of all cells, and, as we shall see later on, of demyelination of nerve fibres.

GAUCHER'S DISEASE

There is a group of disorders of familial nature in which there is abnormal metabolism and accumulation of cerebrosides. Gaucher's disease is the most important example and is characterised by the accumulation of kerasin in the reticulo endothelial system in spleen, lymph nodes, liver, bone marrow and elsewhere. Kerasin is a cerebroside, a normal constituent of nerve tissue and consists of lignoceryl sphingosine combined with galactose. In addition to this galactocerebroside, there is in Gaucher's disease storage of an abnormal gluco-cerebroside not found elsewhere. The nature of the disorder of cerebroside metabolism in Gaucher's disease is unknown. However, lignoceryl sphingosine normally combines with choline to form the important substance sphingomyelin. It has been suggested that if this reaction were defective due to a genetic fault there would be an excess of lignoceryl sphingosine free to combine with galactose and glucose to form galacto- and gluco-cerebroside in abnormal quantities.

REFERENCES

Bernhard, K. and Scheitlin, E. (1952) : *Helv. Physiol. Pharm. Acta., 10:* 523.

Best, C. H. and Huntsman, M. E. (1932) : *J. Physiol., 75:*405.

Best, C. H., Lucas, C. C. and Ridout, J. H. (1953) : *Ann. N. Y. Acad. Sci., 57:*646.

Billing, B. H., Conlon, H. J., Hein, D. E. and Schiff, L. (1953) : *J. clin. Invest., 32:*214.

Cameron, G. R. (1952) : *Pathology of the Cell,* p. 316–328. Edinburgh & London. 1952.

Cornatzer, W. E. and Gallo, D. G. (1956) : *Proc. Soc. exp. Biol. Med., 93:*287.

Frunder, H., Dittrich, H., Lachheim, L. and Miteff, I. (1955) : *Z. Physiol. Chem., 301:*210.

Goebel, A., Friederici, L., Frikas, H. K., Maurer, W. and Nagel, W. (1952) : *Beitr. path. Anat., 112:*36.

Kennedy, E. P. (1957) : *Ann. Rev. Biochem., 26:*119.

Lebedev, (1883) : (See Lubarsch, O., 1897) : *Ergebn. allg. Path. path. Anat., 3:* (i) 631.

Ralli, E. P., Rubin, S. H. and Rinzler, S. J. (1941) : *J. clin. Invest., 20:* 93.

Rosenfeld, (1902) : *Ergebn. Physiol. Abt., 1:* (1) 651.

Rosenfeld, (1903) : *ibid.,* (2) 50.

Snyder, F., Cress, E. A. and Kyker, G. C. (1959) : *J. Lipid Res., 1:*125.

Schulze, G. (1952) : *Dtsch. Z. Verdaunng. Stoffwechselk., 15:* suppl. p. 133.

Thannhauser, S. J. and Reinstein, H. (1942) : *Arch. Path., 33:*646.

Theis, E. R. (1929) : *J. biol. Chem., 82:*327.

Wells, H. G. (1925) : *Chemical pathology*, 5th ed. Philadelphia.

Chapter 3

GLYCOGEN

GLYCOGEN IS PERHAPS the most important storehouse of carbohydrates that we possess. It is a macro-molecular polysaccharide with a tree-like structure. Though found in all cells, including those of placenta and endometrium, its main storage sites are the liver and muscles. Granules of glycogen are found in the leukocytes, cartilage cells and frequently in fat cells (Cameron, 1952). Combinations of protein and glycogen exist in muscle in the form of myosin and myoglobin and with serum globulin. A protein fraction containing glycogen has been obtained from liver.

Under ordinary circumstances the liver contains 0.5 to 6 g. per 100 g. wet tissue of glycogen, varying in amount and in lobular distribution throughout the day and reaching a maximum during the night. Though largely formed from absorbed glucose it is also concerned in gluconeogenesis. In this case fatty acids and proteins are transformed to carbohydrates, first by the production of glycogen and ultimately with the formation of glucose. In this way the blood receives an important gift of sugar independently of that derived directly from the glycogen stores and also by absorption from the intestines. Hepatic glycogen and blood sugar are in equilibrium; when the blood glucose level exceeds approx. 120 mg. per 100 ml. the liver releases less glucose and glycogen synthesis increases. When the blood glucose level falls, more glycogen than usual is broken down by glycogenolysis to replenish the glucose in the blood. This mechanism is controlled by the autonomic nervous system, implemented by hormones from the anterior pituitary, adrenal cortex, thyroid gland and the islets of Langerhans in the pancreas.

THE STRUCTURE OF GLYCOGEN

Since an understanding of glycogen behaviour in the damaged cell depends upon our knowledge of its structure we shall first of all discuss modern ideas on its constitution and then indicate how the giant molecule is built up and broken down according to the demands placed upon it in health and disease.

Glycogen, like amylopectin, the branched fraction of starch, is composed of the monomer anhydro-glucose, existing as units which are joined together by α - 1, 4 glucosidic linkages to form chains, with branches at α - 1, 6 glucosidic linkages.

Three models have been proposed for glycogen:

1. The Staudinger model consists of a long chain of glucoses bound by α - 1, 4 links with unbranched side chains attached to the main chain at branch points by α - 1, 6 links.
2. The Haworth model consists of a number of chains attached one to the next one at branch points by α - 1, 6 links, so that each chain has but one branch point.
3. The Meyer model consists of a combination of the two so that each chain may branch further whether its origin be a 1, 4 link or a 1, 6 link at the branching glucose.

Rabbit liver glycogen, which has been most studied, appears to possess a structure half way between the Haworth and ideal Meyer models.

Glucose is built up into glycogen in the following manner (Cori, 1952-3):

1. The C6 atom of glucose is phosphorylated in all normal cells with adenosine triphosphate (ATP) as donor of phosphorus and hexokinase as enzyme common to all tissues. In this way glucose-6-phosphate is produced through the expenditure of energy and so the energy level of glucose is raised and it is prepared for its many pathways in metabolism.

2. Glucose-6-phosphate is catalysed by the enzyme phosphoglucomutase and Mg ion to glucose-1-phosphate.

3. The enzymes phosphorylase and amylo (1, 4—1, 6) transglucosidase ("brancher enzyme") catalyse glucose-1-phosphate to glycogen. The build up of glycogen thus depends on the activities of the phosphorylase, which makes or breaks the 1, 4 - linkage, and the brancher enzyme amylo (1, 4 — 1, 6) transglucosidase. Phosphorylase lengthens the outer polysaccharide chains until they reach the critical length of at least 8 residues at which they become

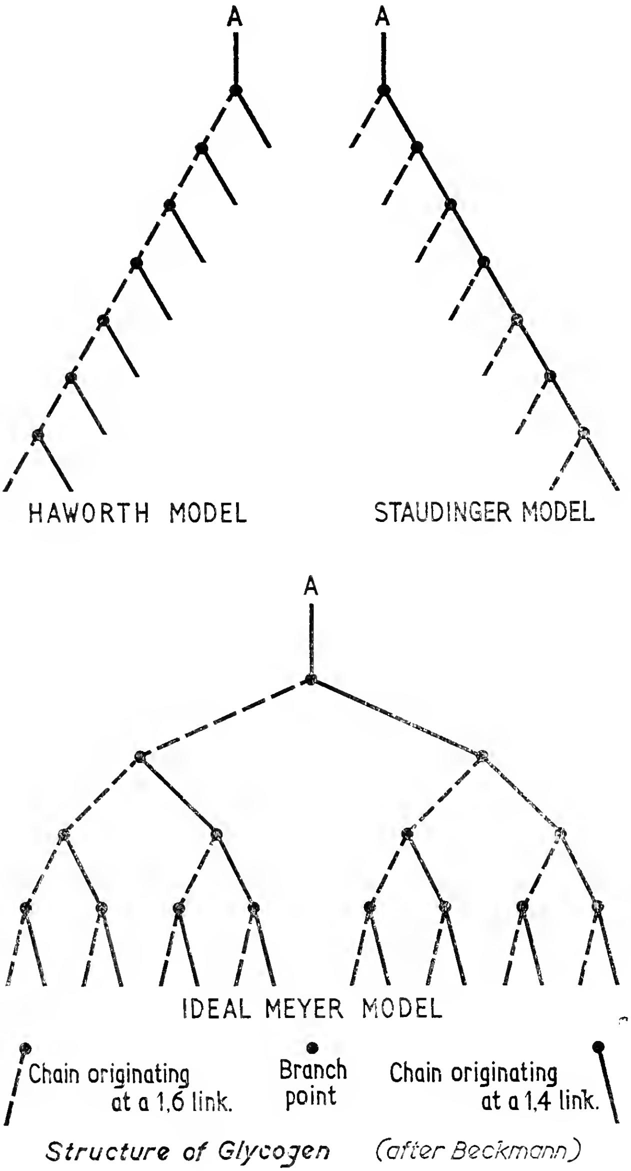

A
A
HAWORTH MODEL
STAUDINGER MODEL
A
IDEAL MEYER MODEL
Chain originating at a 1,6 link.
Branch point
Chain originating at a 1,4 link.
Structure of Glycogen (after Beckmann)

Fig. 2. Proposed Structure of the Glycogen Molecule.

the substrate for the brancher enzyme. The brancher acts on a chain of that length only when it is an outer chain of glycogen. It does not branch short dextrin chains.

Degradation of glycogen is largely brought about by reversed action of these enzymes. Phosphorylase leads to the loss by the chain of one glucose residue and the formation of glucose-1-phosphate. Phosphorylysis stops as the outermost tier of branch points of each molecule is approached since phosphorylase can neither break nor by-pass the branch points. The polysaccharide thus obtained is called "phosphorylase limit dextrin."

A debranching enzyme (amylo - 1, 6 - glucosidase) then removes free glucose from "limit dextrin." Glucose and "limit dextrin" less the outermost tier of branch points are the reaction products of this process. Phosphorylase in the presence of inorganic phosphate can act again on this polysaccharide. Alternating one with the other in this way, the two enzymes degrade the entire glycogen molecule, taking it to pieces tier by tier. Ultimately the digestion mixture contains only glucose and glucose-1-phosphate. In the body tissues the latter is transferred to glucose-6-phosphate through a reversible reaction mediated by phosphoglucomutase. These processes of build up and degradation are depicted in the scheme prepared by Cori (1952-3).

This summary should make clear how the liver maintains blood glucose at normal levels. Through the process of glycogenolysis glycogen is broken down, by the coordinated action of specific enzymes, through glucose-6-phosphate to glucose which is then made available to the blood stream. Glycogen stores are replenished by phosphorylating blood glucose to glucose-6-phosphate which is then transformed to glycogen by hexokinase (Sant' Agnese, 1959).

Somewhere in this scheme must be fitted in the action of hormones. At present we can do no more than offer conjectures based upon experimental studies and await the outcome of future investigations. The pituitary gland exerts an important controlling action on glycogen production in the liver, for that organ almost completely loses its glycogen store when the pituitary is removed. Gluconeogenesis and increased fatty acid synthesis are the main disturbances. Corticotropin (ACTH) and growth hormones deplete glycogen stores in the human liver, but by an unknown mechanism.

Bilateral removal of the adrenal glands decreases hepatic glycogen, with decreased gluconeogenesis if the animal is diabetic. Cortisone increases hepatic glycogen through stimulation of glycogenesis (Young *et al.*, 1948). The action of epinephrine (adrenaline) in inducing raised blood sugar concentration through glycogenolysis has long been known. We discuss the action of insulin later on. The place of sex hormones in carbohydrate metabolism is still uncertain. Thyroid hormone exerts a general effect on metabolism through its influence on the cytochrome mechanism and oxidative phosphorylation in the liver (Maley and Lardy, 1953). Liver glycogen is reduced by giving thyroid extract, partly perhaps as the result of reduction of lactic acid dehydrogenase activity by thyroxin (Vestling and Knoepfelmacher, 1950).

GLYCOGEN-STORAGE DISEASE

Babies are sometimes affected by mysterious diseases in which vast amounts of glycogen are stored within the cells of the liver and kidneys, and on rare occasions in the heart muscle, brain and tongue. The former is often called v. Gierke's disease after the German investigator who described it in 1929. These glycogen storage disorders are not numerically important but they afford a means of studying the structure of glycogen and for this reason they merit close attention. We owe a great debt to Gerty Cori and her collaborators for their brilliant initiation of such studies.

Five types of glycogen storage are recognised though the evidence for some is still scanty (Sant'Agnese, 1959). The most common type follows the classical pattern established by v. Gierke. The liver and at times the kidneys are primarily affected. They usually are greatly enlarged because they are heavily stocked with glycogen which is normal in structure. But the enzyme glucose-6-phosphatase in the liver, and sometimes in the kidneys, is absent or reduced so that the carbohydrate cycle is interrupted. Glycogenolysis does not go on in the usual manner and the child develops hypoglycaemia, ketosis and hyperlipaemia and fails to give a normal glycaemic response after injection of epinephrine.

The cardiac type of generalised glycogen disease—sometimes called Pompe's disease—is much less common, for no more than twenty cases have been recorded. The heart muscle is heavily infiltrated with normal glycogen which interferes mechanically with the muscle fibres and proves fatal in a few months. The glycogenolytic function of the liver is preserved and carbohydrate function proceeds normally. The cause of the defect is unknown. In another closely related variety, the striated muscles are heavily infiltrated with normal glycogen, but the heart muscle escapes. Tests of carbohydrate function are normal; the nature of this condition remains a mystery.

Two varieties of storage disease have been distinguished in which abnormal glycogen is stored in the liver and cells of the reticulo-endothelial system. In one instance, absence of amylo-1,6-glucosidase activity (Debrancher) leads to the production of a glycogen molecule with short, outer branches that resemble a "limit dextrin." In the other form, glycogen possesses few and excessively long outer branches which are thought to be due to inhibition of the branching enzyme. Only one case has so far been reported on which chemical studies have been carried out. The glycogen is similar to the amylopectin of corn and seems to behave as a foreign substance in the body, provoking a fibrous reaction in the liver and kidneys.

GLYCOGEN VARIATION IN DISEASE

A glycogen increase in the cells of the liver, kidneys and heart muscle—occasionally in a great variety of cells—has long been recognised as a characteristic feature of diabetes mellitus. The vacuolated appearance of the β cells of the islets of Langerhans, found in human and experimental diabetes, is now known to be a sign of glycogen accumulation. Frequently the nuclei of liver cells are also affected. The explanation seems to be that the first step of glycogen manufacture—the formation of glucose-6-phosphate—is deficient and glucose piles up in the tissues and blood and overflows into the urine. Studies on liver slices with intact cells from diabetic animals have disclosed two blocks in the metabolism of glucose. One is somewhere between glucose and fructose phosphate though the change of fructose to glucose is not affected (Chernick and Chaikoff, 1951). The other concerns the incorporation of two carbon compounds into fatty acids. Insulin administration overcomes both blocks. Insulin is also thought to be a factor in the transport of glucose phosphate across the cell membrane, while it is known to decrease the output of glucose from the liver. Excess gluconeogenesis from fat in the liver has also been offered as the explanation of the glycogenic infiltration in that organ (Vallance-Owen, 1952) and different types of diabetes have been suggested, but the position requires further investigation, especially because of the interrelationships that exist between carbohydrate metabolism and endocrine function. One intriguing example of glycogen accumulation occurs in rats given 2:4-dinitrophenol subcutaneously for twenty days. The liver then recalls a glycogen storage disease. It shows decreased acid and alkaline phosphatase, unchanged glucose-6-phosphatase and phosphoglucomutase, and is thought to result from decreased peripheral glucose utilisation as happens with adrenocortical hormones (Fonnescu and Severi, 1955).

Reduced glycogen content or its complete loss is a well known happening in damaged cells. A poor oxygen supply, infection or intoxication are the most potent factors that bring this about, apart from inanition. Most of our information comes from histological observation, however, and little reliable quantitative information is available. When the coronary artery is occluded glycogen disappears from the heart muscle fibres in a few minutes long before there is impairment of muscle capacity and cell death (Caulfield and Klionsky, 1959). This effect is very similar to that resulting from autolysis or systemic anoxia (Merrick and Myers, 1954). So, too, limb ischaemia results in a fall in its muscle glycogen level, but there may be a rise at the same time in liver and breast glycogen, followed later by a fall (Stoner, 1958). Repeated haemorrhage depletes the liver of glycogen (Devos, 1952). Needle biopsy has brought convincing evidence of glycogen depletion in the liver during infective hepatitis and many forms of infection. Experimentalists, too, have shown how rapidly glycogen disappears from the liver and other organs in the presence of carcinogenic dyes, poisons such as chloroform and other organic solvents, thioacetamide, and ethionine (Spain and Griffin, 1957). Indeed, much evidence has accumulated over many years that glycogen is a sensitive indicator of cellular damage, but the mechanism underlying its disturbance has attracted little attention.

REFERENCES

Cameron, G. R. (1952) : *Pathology of the cell,* p. 340–2. Edinburgh & London.

Caulfield, J. and Klionsky, B. (1959) : *Amer. J. Path., 35*:489.

Chernick, S. S. and Chaikoff, I. L. (1951) : *J. biol. Chem., 188*:39; *ibid., 193*:793.

Cori, G. T. (1952–3) : *The Harvey Lectures, 48*:145. New York.

Devos, L. (1952) : *Rev. belg. Path., 21*:445.

Fonnescu, A. and Severi, C. (1955) : *Brit. J. exp. Path., 36*:35.

Maley, G. F. and Lardy, H. A. (1953) : *J. biol. Chem., 204*:435.

Merrick, A. W. and Meyers, D. K. (1954) : *Amer. J. Physiol., 177*:441.

Sant' Agnese, P. A. di (1959) : *Ann. N. Y. Acad. Sci., 72*:439.

Spain, J. D. and Griffin, A. C. (1957) : *Cancer Res., 17*:200.

Stoner, H. B. (1958) : *Brit. J. exp. Path., 39*:635.

Vallance-Owen, J. (1952) : *J. clin. Path., 5*:42.

Vestling, C. S. and Knoeppelmacher, A. A. (1950) : *J. biol. Chem., 183*: 63.

Young, N. F., Abels, J. C. and Homburger, F. (1948) : *J. clin. Invest., 27*: 760.

Chapter 4

PROTEINS

THE PROTEINS OF THE NORMAL CELL

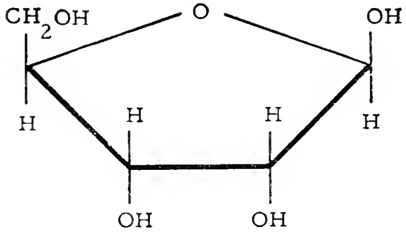
CELL PROTEINS FALL into 2 main categories:

1. The nucleoproteins.

2. Proteins located mainly in the cytoplasm and cytoplasmic particles. The former were originally thought to be specific for the nucleus, but are now known to be important components of the cytoplasm. They consist of nucleic acids combined with other proteins as nucleoproteins. Nucleic acids are built up by condensation of nucleotides which in turn consist of a pyrimidine derivative such as cytosine, linked to a pentose sugar, e.g. ribose, which is linked to phosphoric acid. Successive nucleotides are joined together through the phosphoric acid residues to form nucleic acids as depicted in the figure.

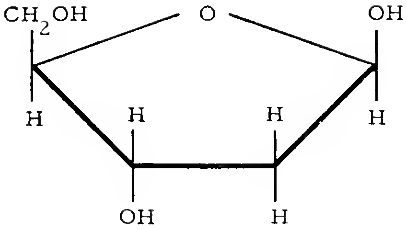
The nucleic acids found in nature are classified as:

1. *Ribonucleic acid* (RNA), which contains d-ribose as its sugar.



Much of the cytoplasmic nucleic acid is RNA which is stored in regions of the cell that are intensely basophilic to staining. The Nissl bodies of nerve cells and the ergastoplasm are such sites and microsomes in general are heavily loaded with RNA. The nucleolus is also a reservoir for nuclear RNA. In all such locations the RNA is probably combined with protein.

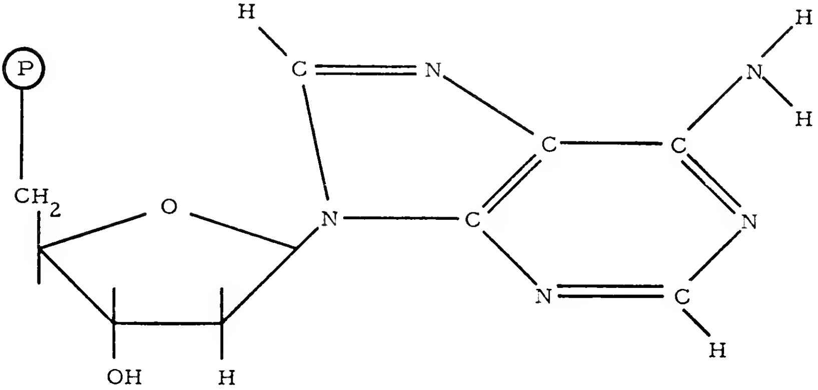
2. *Deoxyribonucleic acid* (DNA) which contains d-2-deoxyribose as its sugar.



Much of the nucleic acid obtained from nuclei belongs to this type with physico-chemical properties that suggest that it is enclosed within a core of proteins of the histone or protamine variety.

Four deoxynucleotides are commonly found in samples of DNA obtained from all sorts of cells.

i. *Deoxyadenylate* (deoxyadenosine 5'phosphate) which is a purine base linked by a glycoside bond to 2-deoxyribose. It occurs in the form of a furanose ring and lacks an oxygen at the 2-carbon position. Esterified at carbon 5 is a phosphate residue.



ii. *Thymidine 5'-phosphate* (thymidylate) which is composed of a pyrimidine-thymine linked as in the case of adenine to a 3 deoxyribose 5'-phosphate. The methyl group on the 5 carbon distinguishes thymine from uracil which is also a pyrimidine. Uracil is a component of RNA and of coenzymes prominent in carbohydrate metabolism.

iii. *Deoxyguanylate,* a purine compound.

iv. *Deoxycytidinate,* a pyrimidine compound.

Before we take a look at protein synthesis we must establish the general setting for each of the varieties of protein. In so doing some overlap into the chemistry of genetics is inevitable. We commence with some remarks about the nucleoproteins.

DNA is now known to be the chemical foundation of the chromosomes, and in this way the key to protein behaviour is the cell. Chromosomes are "undeniably strings of genes" (Lederberg, 1960) and we must therefore look for the secret of gene behaviour in its DNA which conceals the intricate chemical code that governs the heredity of the cell. When a cell divides, its strands of DNA molecules separate to form new chains which are allotted to each of the daughter cells. By a replication process DNA provides the daughter cells with "a complete set of instructions for their own complex development and that of countless future generations of their progeny" (Kornberg, 1959). Its molecule is not being constantly broken down and resynthesised but apparently exists as two helical chains each coiled around the same axis. Each chain consists of phosphate groups joining the DNA residues; lying perpendicular to the axis of the helices are the purine and pyrimidine groups which link by H bonds. The basic protein chains of the nucleoprotein molecule probably wind round with the helix, being electrostatically attracted by the phosphate residues in the polynucleotide backbone.

But DNA is something more than a blueprint. Genetical investigations suggest that the linear genetic information in DNA is translated into linear information in the closely similar but smaller RNA molecules that are available in the nucleus and that this RNA directly controls protein synthesis in the cytoplasm. DNA behaves as a kind of template for manufacturing the cell's structural proteins and the associated enzymatic machinery that decides all of the details and intimate behaviour of these proteins. Each strand of DNA lays down along its length a series of small RNA nucleotides as a set of working dies identically coded for beads of amino acids attached to RNA in a manner which we must now discuss.

Much uncertainty still exists about the way in which proteins are built up in the cell. In all probability, amino acids, and not peptides as was formerly assumed, are the prime units from which the highly complex protein molecules are constructed. These are drawn upon mainly from the free amino acid "pool" of the cell and by means of enzyme activity are united with ATP through their carboxyl group (Hoagland, 1955). The ATP provides energy for the synthesis and comes largely from the mitochondria. In this way an activated amino acid adenylate is produced. The enzymes concerned are distributed in the soluble fraction (cell sap) of many, if not all, cells though some activity also may be associated with particulate cell structures such as the microsomes. There may be other ways of "activation" of amino acids but these are little more than conjecture at the present time (Wieland and Pfleiderer, 1957—review). Uncertainty exists, too, about the role of vitamin B_{12} in protein biosynthesis; it seems to be true enough that amino acids are incorporated into protein more slowly when there is a shortage of B_{12}.

Activated amino acids are next bound to the RNA present in a fraction of the cell sap. The latter, known as soluble RNA (SRNA) may well serve as an intermediate stage between activated amino acid and ribonucleoprotein situated in the microsomes (Hoagland *et al.* 1957). Perhaps the reaction goes on in the following manner:

$$\text{Amino acid} + \text{ATP} \rightleftharpoons \text{Amino acid AMP} + \text{PP}$$

$$\text{Amino acid} - \text{AMP} + \text{SRNA} \rightleftharpoons \text{Amino acid} - \text{SRNA} + \text{AMP}$$

There seem to be specific binding sites for each amino acid. At any rate SRNA must have a certain terminal nucleotide sequence before it can act as an amino acid receptor and the amino acids so attached are not present in peptide linkage (Simkin, 1959—review).

Much evidence now exists for the view that the microsomes which are storehouses of ribonucleoprotein are important sites of protein synthesis (Chantrenne, 1958; Loftfield, 1957). They may well be the end of an assembly line which begins in the nuclear DNA and actually manufactures the structural protein of the cell. Here, too, are produced the proteins destined for secretion by the cell, both normal and abnormal, e.g. serum albumin, antibody, Bence-Jones protein, myeloma protein and pancreatic juice protein. The microsomes are not, however, the only site of protein synthesis though they certainly account for a large part in some cells. Mitochondria can build some protein, including cytochrome *c*. But the genetic control is ultimately exerted by DNA. Nevertheless, there is sound evidence that protein synthesis can occur even in the absence of DNA. Indeed, interference with DNA synthesis does not, in general, affect the synthesis of protein or of RNA (Chantrenne, 1958; Fincham, 1959). Though many biochemists like the idea that DNA exerts its control on protein synthesis by passing information on to RNA which then controls protein synthesis directly, the view is not accepted by all and much more work is needed to establish the hypothesis. Taking everything into consideration, it seems certain that RNA is concerned in at least two distinct ways in protein building, (a) through SRNA, as we have seen, it is involved in the transport of activated amino acid residues to the site of protein synthesis, and (b) through ribonucleoprotein, it is involved at the site of synthesis (Simkin 1959), Whatever may be the intermediate stages there is no doubt that RNA must be present at the site of synthesis and it is generally assumed that this RNA assists in the linkage of amino acid residues to form polymers and in particular in determining the sequence of amino acid residues in the polypeptide chains so formed. We have no direct information about the mechanisms involved although many ingenious suggestions have been offered. Crick (1958), for instance, thinks that SRNA might provide the activated amino acid with an "adaptor" molecule, the adaptor then playing some part in controlling the way in which the amino acids are polymerised together in the correct sequence. Hoagland suggests that the whole of the SRNA molecule might serve as an adaptor. Crick rather fancies a nucleotide. In any case, the existence of a "template" appears to be necessary for polymerisation to form a polypeptide chain (Fincham).

PROTEIN DISTURBANCES IN THE DAMAGED CELL

We are on even more shaky ground in assembling facts about protein disturbances in the damaged cell than we were in discussing protein metabolism in the normal cell. Clearly, the most that we can do is to put before the reader such information as we possess at present, knowing full well that much of what we write may be superseded before very long.

Cloudy Swelling and Hyaline Droplets

Elsewhere one of us has marshalled evidence for regarding this common variety of reversible cell injury as the outcome of upset of water, salt and protein metabolism (Cameron, 1956). The argument, so far as it concerns protein upset, is based on experiments devised by Spector (1954) and by Oliver and his colleagues (1954). In searching for the sources of the urinary protein in nephrosis, Spector showed that homologous blood proteins labelled with ^{131}I are taken up in increased amounts by the tubule cells of nephrotic rats and stored in the mitochondria and microsomes. These proteins had been filtered out in large amounts by the glomeruli and excreted in considerable quantities in the urine. About four to ten times as much protein as normal was found in the tubule cells which means that these cells were storing protein vigorously. At this time, they show the microscopical picture of cloudy swelling. Under such circumstances cloudy swelling may well be the outcome of "accelerated function leading to protein storage." We are reminded of the suggestion made long ago by Rudolf Virchow who attributed the cloudy swelling in a kidney undergoing compensatory hypertrophy after the loss of the other kidney to functional over-activity.

Oliver and his colleagues have followed the development of granules and droplets in renal tubule cells of rats given rat or foreign protein parenterally and in patients with proteinuria and tubular damage. They, too, find that a close relationship exists between the formation of such granules and mitochondrial disturbance. In cells in which droplets are forming, mitochondria become shortened and thickened, eventually take on club-like forms and are masked by the new droplets, all of which stain by specific mitochondrial methods. Oliver concludes that proteins passing through the glomerular filter are in part absorbed by the epithelial cells of the proximal convolutions without any apparent alteration of the cytological pattern unless the absorptive capacity is overruled either by the amount or nature of the protein or by disturbance of tubular cell function. When the absorptive powers of the tubules are exceeded the mechanism of droplet formation is brought into play and the cell assumes an appearance indistinguishable from cloudy swelling. Similar changes, often termed hyaline change or degeneration, are known to occur in human subjects during the excretion of haemoglobin, Bence-Jones protein and myeloma proteins, and indeed in any heavy proteinuria due for example to glomerulonephritis.

Hypertrophy of Cells

When portion of a rat's liver is removed the part left behind hypertrophies and inclusion bodies appear within the rapidly regenerating liver cells. These are mainly albuminous in nature. A critical liver mass and plasma albumin must be reached before they become apparent in the liver cells, and their development is prevented by pre-operative starvation which lowers the plasma albumin level. Feeding with glucose will do the same (Doniach and Weinbren, 1952). The basophilic bodies of liver cells that we know to be masses of ribonucleoprotein are greatly increased during gestation (Clavert and Assenmacher, 1952). A similar increase of RNA and also of DNA occurs at an early period of compensatory hypertrophy in the liver along with increase in activity of a number of cell enzymes, especially esterases and phosphatases. Such facts suggest an increase in protein synthesis without necessarily a potentiation of all of the cellular biochemical activities correlated with it (Pileri *et al.*, 1957). No doubt a similar explanation holds for the liver cell hypertrophy of Waldenström's macroglobulinaemia with its cytoplasmic increase of RNA (Wuketich and Siegmund, 1958). Nucleic acids are increased, too, in hypertrophying renal cells; here, too, RNA is affected more than DNA (Vegni, 1953; Fautrey *et al.*, 1955). Increase of protein N is remarkable at the height of hypertrophy, especially in the microsome fraction of the cells (Dianzani and Biaggini, 1954).

ACTION OF HORMONES ON CELL PROTEINS

When the pituitary gland is removed from the rat the RNA content of the mitochondrial fraction of the liver decreases and the yield of the microsome fraction declines. Growth hormone restores to normal the mitochondrial RNA and leads to an apparent shift of RNA from the microsome fraction to the supernatant fraction, i.e. to other portions of the cytoplasm. Untreated adrenalectomised rats acquire more liver DNA but lose some of their mitochondria. Alloxan diabetes in the rat increases the yield of supernatant liver fraction at the cost of other components. Castration produces no change in the liver fractions (Reid, 1956).

Oestrogens lead to doubling of the nuclear volume in the rat uterine gland cells and tripling in volume of surface epithelial cell nuclei. The DNA content per nucleus remains constant, the water content per nucleus is slightly increased and there is a substantial increase in total organic matter per nucleus. The non-histone protein fraction of the nucleus is mainly responsible for the nuclear hypertrophy (Gelfand and Clemmons, 1955).

An increase of DNA has been recorded in the liver cells of patients dying as a consequence of a thyroid crisis after thyroidectomy for Graves' disease, possibly the result of toxic action of thyroxin (Majewski, 1956). Although the meaning of these isolated facts is far from clear, they point the way to further investigations that may well throw much light on protein synthesis.

MALNUTRITION

Rats fed on low protein (6.4 per cent), high carbohydrate diet, comparable with that of the poor people in Jamaica, show great reduction in N:DNA ratio of the liver and still more of muscle. Re-feeding quickly leads to new formation of protein and DNA in the liver at a rate about twice as speedy as during normal growth (Mendes and Waterlow, 1958).

Kwashiorkor, a disease caused by protein deficiency, in the presence of excess dietary carbohydrate, is widely distributed over Africa, West Indies, Ceylon. Children so affected show an enlarged liver which is fatty and seriously depleted of protein. The average loss of liver protein and RNA is 40 per cent in Jamaican cases. The muscles lose more protein than the liver (Waterlow and Weiss, 1956).

AMYLOID INFILTRATION

This mysterious condition presents a challenge that has defied investigators for many years. The composition of amyloid is still not known, although it probably consists of proteins and sulphated mucopolysaccharides akin to heparin and it possesses a micellar structure. When animals are injected for a long time with bacteria or their products, foreign proteins such as casein, ribonucleate or antitoxin, they develop amyloid disease. While this is happening the blood undergoes a striking dysproteinaemia characterised by increase of serum β globulin and a fall in albumin. Serum hexosamine also increases. Amyloid disease can be made to disappear; the β globulin level falls as the amyloid is resorbed (Richter, 1956; Giles and Calkins, 1958). But what lies behind these disturbances has still to be discovered. Some workers believe that disturbed function in the cells of the reticulo-endothelial system cause the blood changes and the associated amyloid infiltration of organs such as the liver, kidney, spleen and intestine.

DISTURBANCES DUE TO TOXIC ACTION ON CELLS

We have already discussed the best investigated examples of this group and now add a few more instances. Carbon tetrachloride, hepatotoxic serum and x-rays increase the cytoplasmic β globulins when their action is mild but lead to a decrease with an increase in γ globulins when severe (Demling, 1954). Carbon tetrachloride induces a considerable rise in RNA and a smaller increase in DNA in the liver of rats fed on protein-free or 23 per cent casein diet in forty-eight hours when regeneration is going on actively. The RNA increase is much less in fasting animals (Campbell and Kosterlitz, 1952). Spectrophotometric analysis discloses a build up of DNA in the nucleus before the cell divides and persistent abnormal nuclear masses after regeneration is complete (Hoffmann *et al.*, 1955). A marked decrease in RNA has been recorded in the liver cells of dogs damaged by administration of phosphorus (Barone and Parisi, 1954). We have noted elsewhere the apparent affinity of triethyltin for liver proteins and the interesting suggestions that may arise from this demonstration. Similar affinities between carcinogenic agents, especially certain azo dyes and thioacetamide, and cell proteins are known to exist, but their significance is still obscure (Miller and Miller, 1947; Hultin, 1956; Novikoff, 1957; Grant and Rees, 1957) .

HAEMOCHROMATOSIS

This is a genetically determined disease in which the basic disorder is an abnormally high level of absorption of iron with subsequent deposition of an iron-protein complex in liver, skin, pancreas and elsewhere. The iron-protein complex resembles but is not identical with, haemosiderin. There is extensive damage to the affected organs, partly due to cell death and replacement fibrosis, partly due perhaps to a toxic action of iron on glycolysis, triose phosphate dehydrogenase being especially vulnerable to the damaging effects of iron ions.

The absorption of iron from the intestine is governed normally by a system of proteins. The first of these, apoferritin, is present in the mucosal epithelium and complexes with iron in the bowel contents to form the second protein, ferritin. Depending on the body's needs, ferritin gives up its iron to a third protein, transferrin, that transports iron in the blood stream. Normally, when all the apoferritin in the bowel mucosa has combined with iron, absorption of the metal ceases. In haemochromatosis, however, the mechanism is distorted and iron continues to be absorbed so that the blood transferrin is permanently saturated with iron. The precise nature of the disturbance has yet to be identified. It seems likely, however, that the defect lies in the transfer of iron from ferritin to transferrin probably due to congenital absence of some regulating factor.

It is possible that in addition to the above abnormalities, patients suffering from haemochromatosis exhibit a more general nutritional defect which may contribute to the pathology of the condition, notably hepatic fibrosis. Animal experiments indicate that protein metabolism may also be concerned in this aspect of the disease together perhaps with a relative choline deficiency.

HEPATO-LENTICULAR DEGENERATION

This is a genetically determined disease with some similarities to haemochromatosis. Copper is normally transported in the body in firm combination with a copper-binding globulin called caeruloplasmin. In hepato-lenticular degeneration there is a deficiency of this protein, and copper is transported instead in loose combination with plasma albumin. As some tissues appear to have a greater affinity for copper than does albumin, the metal becomes deposited in excessive amounts in liver and brain with resultant fibrosis and degeneration. There is also damage to renal tubules with glycosuria and aminoaciduria.

ABNORMALITIES OF PLASMA GLOBULINS

A large portion of the plasma globulins is synthesised in the cells of the reticulo-endothelial system and disease of this system is apt to be reflected in abnormalities in the globulin fraction of the serum. Thus in agammaglobulinaemia, a disease characterised by deficiency of circulating γ globulin, there is a genetically-determined deficiency of plasma cells. These cells play a vital role in the production of γ globulin and therefore of antibodies. Because of their inability to produce antibodies patients suffering from this condition are abnormally susceptible to infection.

In certain neoplastic diseases of the reticulo-endothelial system there may be abnormal synthesis of globulin-like proteins. In myelomatosis, in which the neoplastic cells resemble plasma cells, there may be abnormal synthesis of a low molecular weight protein (Bence-Jones protein) which is excreted freely in the urine. There may also be abnormal globulins of large molecular size in the circulation. In leukaemia (neoplasia of blood leucocytes) similar defects may exist such as the synthesis of abnormally large globulin molecules (macroglobulinaemia). Similar dysproteinaemia may develop in non-neoplastic diseases such as disseminated lupus erythematosis. In a variety of conditions, some inflammatory, some degenerative, a new protein appears in the circulation (C-reactive protein) distinguished empirically by its ability to react with pneumococcal antigens. Other reactive proteins, possibly with more specific characteristics, have been observed in the circulation in other diseases, notably rheumatoid arthritis. The significance of many of these findings, however, has not yet been fully revealed.

REFERENCES

Barone, G. and Parisi, A. (1954) : *Patol. Sper., 42:*182.

Cameron, G. R. (1956) : *New pathways in cellular pathology,* Chap. 8. London.

Campbell, R. M. and Kosterlitz, H. W. (1952) : *Brit. J. exp. Path., 33:* 518.

Chantrenne, H. (1958) : *Ann. Rev. Biochem., 27:*35.

Clavert, J. and Assenmacher, I. (1952) : *C. R. Acad. Sci. Paris, 234:*471.

Crick, F. H. C. (1958) : *Sympos. Soc. exp. Biol.,* No. 12, p. 138. Lond.

Demling, L. (1954) : *Gastroenterol., 81:*129.

Dianzani, M. U. and Biaggini, G. C. (1954) : *Arch. Sci. Biol., 38:*582.

Doniach, I. and Weinbren, K. (1952) : *Brit. J. exp. Path., 33:*499.

Fautrez, J., Cavalli, G. and Pisi, E. (1955) : *Nature,* Lond., *175:*684.

Fincham, J. R. S. (1959) : *Ann. Rev. Biochem., 28:*343.

Gelfand, S. and Clemmons, J. J. (1955) : *J. cell. comp. Physiol., 46:*529.

Giles, Jr., R. B. and Calkins, E. (1958) : *J. clin. Invest., 37:*846.

Grant, H. C. and Rees, K. R. (1957) : *Proc. Roy. Soc. B., 148:*117.

Hoagland, M. B. (1955) : *Biochim. Biophys. Acta, 16:*288.

Hoagland, M. B., Zamecrik, P. C. and Stephenson, M. L. (1957) : *Biochim. Biophys. Acta, 24:*215.

Hultin, T. (1956) : *Exp. cell Res., 10:*71, 697.

Hoffmann, T., Haines, M. B., Lapan, S., Riszki, R. and Post, J. (1955) : *Arch. Path., 59:*429.

Kornberg, A. (1959) : *The Harvey Lectures, 53:*83. New York.

Lederberg, J. (1960) : *Science, 131:*269.

Loftfield, R. B. (1957) : Progress in biophysics and biophysical chemistry. *8:*347.

Majewski, C. (1956) : *Patol. Pol., 7:*257.

Mendes, C. B. and Waterlow, J. C. (1958) : *Brit. J. Nutrition, 12:*74.

Miller, E. G. and Miller, J. A. (1947) : *Cancer Res., 7:*468.

Novikoff, A. B. (1957) : *Cancer Res., 17:*1010.

Oliver, J., MacDowell, M. and Lee, Y. C. (1954) : *J. exp. Med., 99:*589.

Pileri, A., Camurati, P. and Gavosto, F. (1957) : Tumori, *43:*137.

Reid, E. (1956) : *J. Endocrin., 13:*319.

Richter, G. W. (1956) : *J. exp. Med., 104:*847.

Simkin, J. L. (1959) : *Ann. Rev. Biochem., 28:*145.

Spector, W. G. (1954) : *J. Path. Bact., 68:*187.

Vegni, L. (1953) : *Arch. Sci. biol., 37:*454.

Waterlow, J. C. and Weiss, T. (1956) : *J. clin. Invest., 35*:346.

Wieland, T. and Pfleidered, G. (1957) : *Advances in Enzymology, 19:* 235.

Wuketich, S. and Siegmund, G. (1958) : *Frankfurt Z. Path., 69*:62.

Chapter 5

ENZYMES

RESPIRATION AND THE SUPPLY OF ENERGY

THE INTEGRITY OF THE cell, its continued existence and the maintenance of its specialised functions depend ultimately on a supply of energy. Interference with this supply occurs as a result of a large variety of injuries including toxic substances, some of which are discussed elsewhere, and if sufficiently severe lead to cell death (necrosis).

The vital supply of energy is produced by the cell itself and is derived from the metabolism of carbohydrate, fat and protein. In effect, the energy is derived from the conversion of glucose to pyruvate (glycolysis) and the oxidation of pyruvate to carbon dioxide and water. Glycolysis provides about 20 per cent of the cell's energy requirements and the oxidation of pyruvate the remainder.

To release the energy of glucose and pyruvate under the ordinary conditions of the chemical laboratory would require high temperatures or extremes of hydrogen ion concentration. The body does so at 37° and at physiological pH. This is achieved with the aid of enzymes, which are very efficient organic catalysts usually protein in nature and characteristically specific for the reaction they control. Again, energy liberated by a usual chemical reaction *in vitro* is obtained in the form of heat. Energy in this form would be useless to the cell which instead possesses a specialised compound, adenosine triphosphate (ATP) capable of trapping energy (in the shape of high energy phosphate bonds) and passing it on to those reactors which require it. Finally, to allow oxidation to occur under physiological conditions, the cell does not add oxygen to the substance being oxidised, but instead subtracts hydrogen ions. The hydrogen ions (i.e. electrons) are passed on to specialised compounds with an affinity for hydrogen known as hydrogen carriers. Cytochrome, flavoproteins and pyridine nucleotides are the most important of these compounds. The process is known as electron transport and occurs in a stepwise fashion, the final step being the transport of electrons from cytochrome to oxygen. In the process the cytochrome, of course, becomes oxidised and thus becomes available for further service as a hydrogen carrier (Fig. 3).

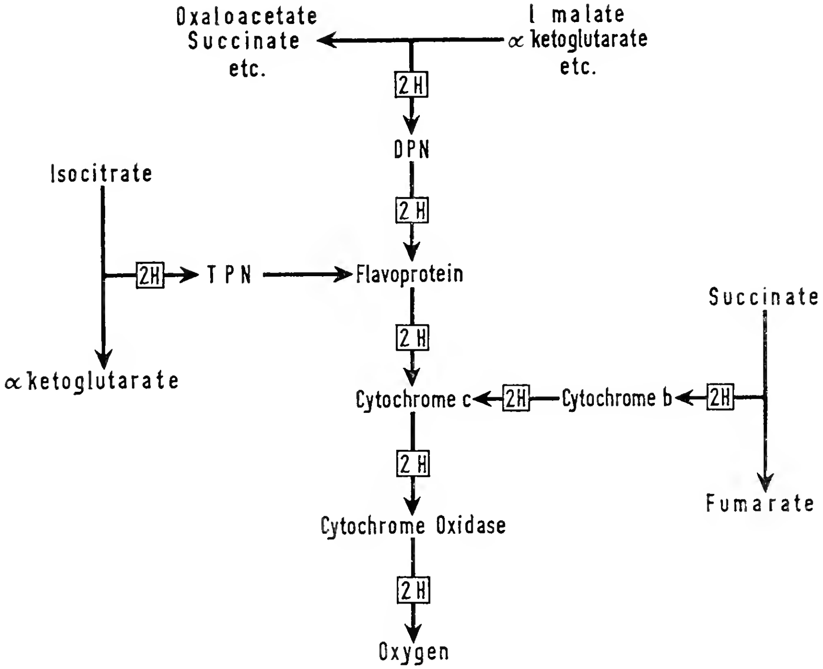

Oxaloacetate
Succinate
etc.
l malate
α ketoglutarate
etc.
2 H
DPN
2 H
Isocitrate
2H
TPN
Flavoprotein
α ketoglutarate
2 H
Succinate
Cytochrome c
2H
Cytochrome b
2H
2 H
Fumarate
Cytochrome Oxidase
2 H
Oxygen

Fig. 3. The Electron Transport Chain.

The supply of energy is generated in the following manner (Baldwin, 1957). It is convenient to take glucose as a starting point (Fig. 4). The sugar is phosphorylated and converted to triose phosphate. This substance is then broken down to phosphoglyceric acid with the liberation of energy which is stored as an energy-rich phosphate bond in the molecule of ATP. Phosphoglyceric acid is then converted to pyruvate with the liberation of another parcel of energy, also stored in the ATP molecule. This is glycolysis. There exists an alternative pathway for the breakdown of glucose termed the hexose monophosphate shunt that may be of particular importance in the synthesis of ribonucleic acid (RNA) (Fig. 5).

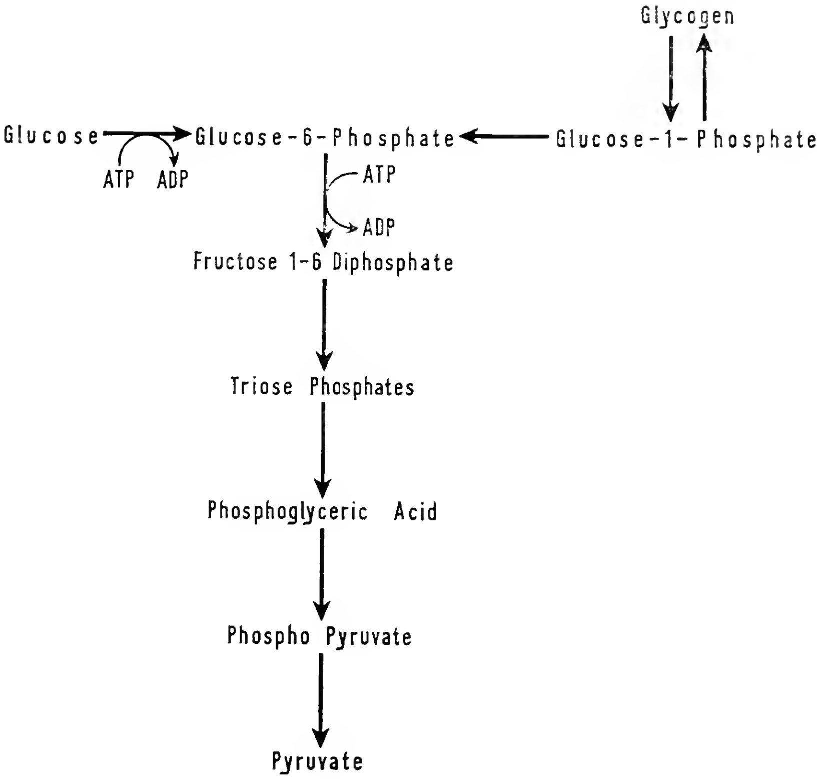

Glycogen
Glucose
ATP
ADP
Glucose-6-Phosphate
Glucose-1-Phosphate
ATP
ADP
Fructose 1-6 Diphosphate
Triose Phosphates
Phosphoglyceric Acid
Phospho Pyruvate
Pyruvate

Fig. 4. Diagrammatic Representation of Glycolysis.

The major part of the cell's energy comes, however, from the further breakdown of pyruvate to CO_2 and water. The first step is the conversion of pyruvate to acetyl CoA, i.e. a complex between the remains of the pyruvate molecule and coenzyme A. Electrons lost in the process are transferred to diphosphopyridine nucleotide (DPN). Acetyl CoA then condenses with oxaloacetate to form citrate which in turn loses electrons to triphosphopyridine nucleotide (TPN) and also loses a carbon atom to become α-ketoglutarate. The process is repeated and α ketoglutarate is converted to succinate which loses electrons to flavoprotein β with the aid of succinic dehydrogenase and is converted to fumarate and then malate, and then by malic dehydrogenase to oxaloacetate. The reactions then continue in a cyclical fashion (the tricarboxylic acid cycle). In one revolution of the cycle 10 hydrogen atoms have been removed and transferred to oxygen by way of the hydrogen carriers, three carbon atoms have been removed and one molecule of pyruvate has been completely oxidised to CO_2 and H_2O (Fig. 6).

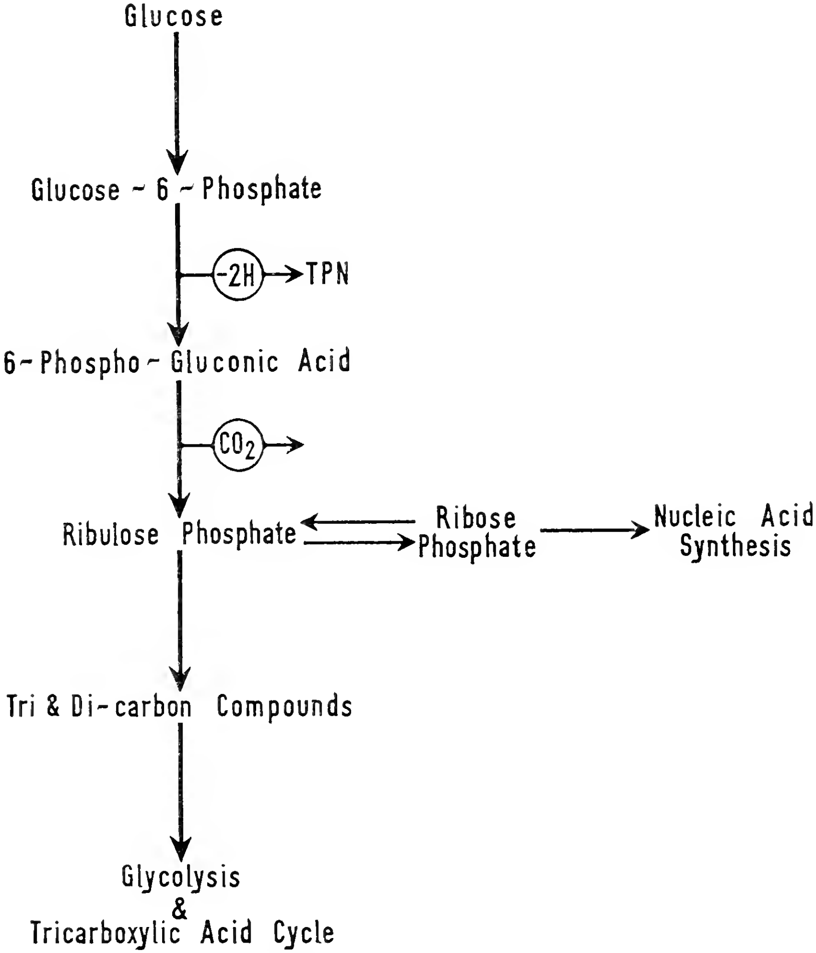

Glucose
Glucose ~ 6 ~ Phosphate
-2H
TPN
6~Phospho~Gluconic Acid
CO2
Ribulose Phosphate
Ribose Phosphate
Nucleic Acid Synthesis
Tri & Di-carbon Compounds
Glycolysis & Tricarboxylic Acid Cycle

Fig. 5. The "Shunt" or Pentose Phosphate Pathway of Glucose Breakdown.

Energy is liberated at each oxidative step of the cycle, i.e. wherever electrons are transferred. As in glycolysis the generation of energy is associated with the synthesis of ATP from ADP. A molecule of ATP is synthesised every time a pair of electrons is transferred from one hydrogen carrier to another. This process of energy production by way of oxidation and ATP synthesis is known as oxidative phosphorylation.

Although glucose (& therefore by implication carbohydrate in general) was chosen as a starting point, both fats and proteins feed the respiratory pathway just described. Fatty acids (derived from neutral fats) are converted to acetyl CoA by β oxidation. Amino acids (derived from proteins) are converted to the corresponding keto acid by deamination or transamination. Thus glutamic acid becomes α ketoglutaric acid and alanine becomes pyruvic acid. The advantage of the cyclical system is that any of the carboxylic acids which act as intermediate substrates can be used by the cell to maintain oxidative phosphorylation. Another point worth making is that the most basic of metabolic processes, respiration (the uptake of oxygen and the formation of carbon dioxide) is essentially a device for generating the energy needed to maintain all the multiple processes that together constitute life. The cell achieves this by synthesising energy-rich ATP in exchange for acetyl CoA. Each step in the long series of reactions leading to this end is catalysed and depends upon one of the respiratory enzymes.

THE EFFECTS OF INJURY ON RESPIRATION, GLYCOLYSIS AND OXIDATIVE PHOSPHORYLATION

The effects on respiratory enzymes and the reactions they control of injury in the general sense are best considered by a study of events in cells which are "dying" because they have been removed from the living animal. It is known that death of cells is accompanied by the dissolution of their structure (autolysis) and that this change is associated with activity of cellular proteolytic enzymes and with a rise in intracellular acidity, phenomena which are considered elsewhere (see under Autolysis).

The sequence of events has been examined more carefully by perfusing the rat liver following its removal from the body

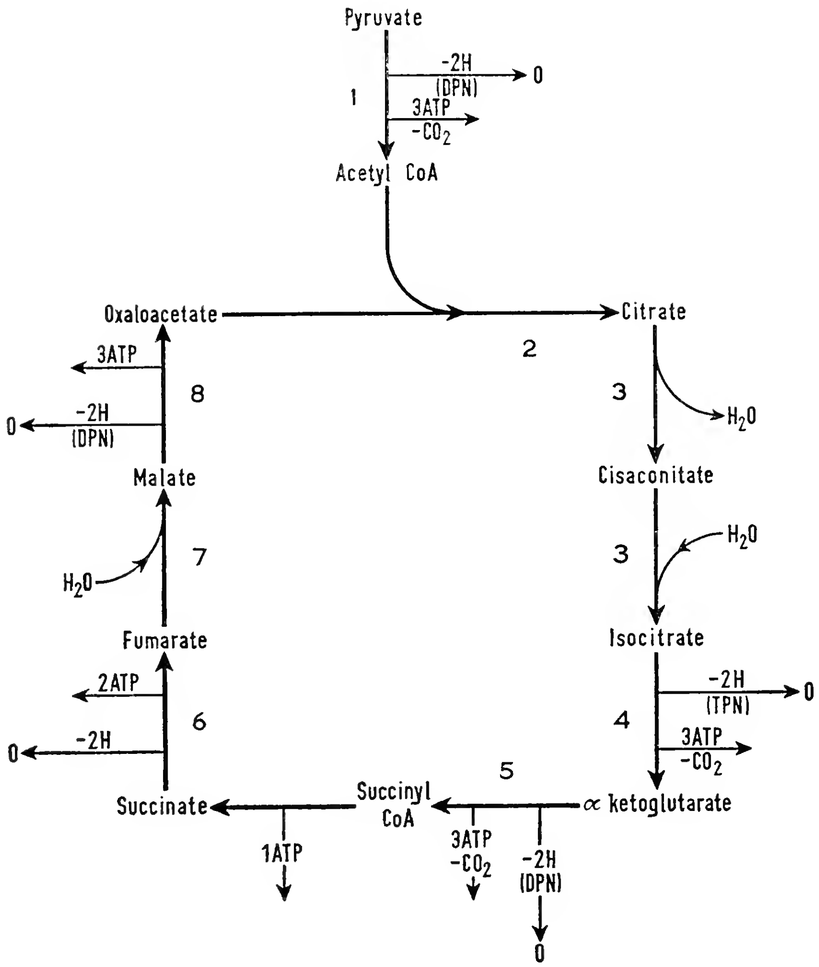

Pyruvate
-2H
(DPN)
0
1
3ATP
-CO_2
Acetyl CoA
Oxaloacetate
Citrate
2
3ATP
8
-2H
(DPN)
0
3
H_2O
Malate
Cisaconitate
H_2O
7
3
H_2O
Fumarate
Isocitrate
2ATP
6
-2H
0
-2H
(TPN)
0
4
3ATP
-CO_2
5
Succinate
Succinyl CoA
α ketoglutarate
1ATP
3ATP
-CO_2
-2H
(DPN)
0

Fig. 6. The Tricarboxylic Acid (TCA, Krebs) Cycle.

1. Pyruvic oxidase.
2. Condensing enzyme.
3. Aconitase.
4. Isocitric dehydrogenase.
5. α ketoglutaric oxidase.
6. Succinic dehydrogenase.
7. Fumarase.
8. Malic dehydrogenase.

(Dawkins *et al.,* 1959) . The basic injury here consists in depriving the cell of its blood supply.

An isolated unperfused liver rapidly loses its ability to oxidise the substrates of the tricarboxylic acid (TCA) cycle. The oxidation of succinate by succinic dehydrogenase is an exception, and is maintained for some time. This failure of oxidation is at least partly due to loss of the coenzymes, e.g., DPN and TPN which, as described above, are closely involved in respiration. The importance of these substances is shown by the ability of added DPN to maintain oxidation of substrates such as pyruvate. It is of interest, too, that oxidation of succinate to fumarate does not require the presence of the pyridine nucleotides as coenzymes. Even more rapid than failure of oxidation is the loss of oxidative phosphorylation, i.e. the synthesis of ATP. This change occurs within a few minutes of incubating the isolated liver at 38°. Other abnormalities that develop are loss of potassium and intracellular accumulation of sodium, calcium and water.

The early failure of oxidation and oxidative phosphorylation may be prevented by perfusion of the liver with glucose or with a tricarboxylic acid cycle substrate such as glutamate. In the presence of glucose oxygen is not required.

The interpretation of these experiments is not simple but it seems likely that the basic and earliest failure is loss of the ability to synthesise ATP due to lack of substrates for the processes of glycolysis and oxidation and to loss of the coenzymes also required for these complex reactions. Inability to synthesise ATP leads to failure of oxidative phosphorylation and thus to loss of the cell's supply of energy. This is followed in turn by further loss of coenzymes due to inability to synthesise them, by profound disturbances in water and electrolyte movements and by a general failure of those reactions which depend upon ATP for an energy supply e.g., the synthesis of proteins and phospholipids.

Mitochondria isolated from liver cells, and then incubated *in vitro* show degenerative changes similar to those observed in the parent tissue. Mitochondria are the chief intracellular seat of respiratory activity and of the associated generation of energy. On incubation they show a rapid loss of oxidative phosphorylation which may be prevented by the addition of oxidisable substrate. Similarly, in isolated mitochondria damaged by contact with high concentrations of calcium, loss of oxidative phosphorylation may be prevented by the addition of ATP and of DPN.

It is of interest that although the addition of glucose will allow oxidative phosphorylation to proceed in liver cells isolated from their blood supply, even in the absence of oxygen, the level of ATP in the cells falls progressively unless oxygen too is supplied. This observation confirms the inability of glycolysis alone to maintain energy generation and ATP synthesis. Another interesting fact is that the level of -SH groups in the cell (chiefly glutathione) falls rapidly in an isolated liver supplied with oxygen and less rapidly under anaerobic conditions. The nature of this phenomenon is obscure.

Isolated livers supplied with glucose or with oxygen plus an oxidisable substrate maintain metabolic activity for several hours. Eventually, however, they develop the failures described above. This subsequent collapse reveals further weak links in survival. Thus the loss of coenzymes at this stage is partly due to a shortage of their precursors, e.g., adenosine and nicotinamide. Another and probably very important factor influencing survival at this stage is the level of liver glycogen, this substance probably representing the most readily available substrate. The superiority of glycogen over glucose as a means of increasing survival is probably due to the fact that utilisation of glucose requires hexokinase and ATP both of which may be deficient in the cell isolated from its blood supply. The accumulation of intracellular calcium (due to disturbances of electrolyte metabolism) cannot be the cause of eventual deterioration of respiratory activity (calcium is a respiratory poison), since calcium accumulation may be prevented with the aid of promethazine HCl (phenergan) without affecting the respiratory decline. The synthesis of complex molecules is amongst the last of the cell's reactions to be lost. They fail eventually for lack of energy due to loss of ATP synthesis.

It can be seen that primary causes of cell death after loss of blood supply are associated with disturbances of the reactions controlled by the respiratory enzymes and may be listed as failure of ATP synthesis, loss of coenzymes especially DPN and TPN, loss of -SH groups and (for rat liver) loss of liver glycogen. There is in addition disruption of water and electrolyte balance, and secondary to failure of energy supply, failure of a wide range of complex synthetic reactions. Other aspects of this matter are dealt with in the chapter on Autolysis.

SPECIFIC RESPIRATORY POISONS

The behaviour of liver cells removed from the blood supply has been used as an illustration of the vulnerability of the respiratory enzyme system to injury in the broadest sense of the term. There are, however, many poisons and deficiency states known to injure this system by causing a failure at one or more specific sites in the complicated cycle of reactions. Some toxic compounds of this nature associated with characteristic pathological lesions are discussed in the chapter on Toxic Agents. Others will be described briefly here, (see also Hunter, 1955, and Brit. Med. Bull., 1954).

Cyanide

The classical example is cyanide, which in small amounts leads to rapid death. Cyanide acts by specific inhibition of the enzyme cytochrome oxidase which is required for the transfer of electrons from cytochrome to oxygen (Fig. 3). The mode of action of cyanide is to form a complex with the metal prosthetic group which is an essential part of the cytochrome oxidase molecule. As a result, the enzyme is inactivated and all oxidative reactions come to a standstill.

2:4 dinitrophenol

A similar example is provided by 2:4 dinitrophenol (DNP). This substance prevents specifically the synthesis of ATP, while allowing the associated oxidations to proceed (Fig. 6). As a result, the generated energy is dissipated as heat, and is no longer available for the bodily reactions that require it. To compensate for this failure there is a vain attempt to oxidise all available substrate and a consequent rise in basal metabolic rate. Observation of this property without realisation of its significance led to a disastrous utilisation of 2:4 dinitrophenol as a "slimming" compound.

Fluoroacetate

Another classical instance of a respiratory poison acting at a specific site is fluoroacetate, a substance which may cause death in convulsions within a few hours. Fluoroacetate acts by preventing the oxidation of citrate to α ketoglutarate (Fig. 6). It does so by inhibiting the enzyme aconitase which is required for this reaction. Before it can exert this effect, fluoroacetate is first "activated" by the body to form fluoracetyl CoA. This substance then condenses with oxaloacetate and is further metabolised to fluorocitrate which then inhibits competitively the oxidation of citrate. As a result the respiratory cycle and its attendant energy yielding reactions are brought to a standstill. The conversion of harmless fluoracetate to poisonous fluorocitrate was termed by Peters "lethal synthesis."

Carbon Monoxide

Carbon monoxide gas interrupts respiration in a different fashion by combining with haemoglobin and thus preventing this substance from acting as an oxygen carrier. The gas has another property, that of complexing with cytochrome oxidase (Fig. 3) and this may possibly account for some of the later manifestations of non-fatal carbon monoxide poisoning.

Lewisite, Mustard Gas and the Nitrogen Mustards

These compounds were first studied as chemical warfare agents, one of whose effects was to cause blistering and necrosis, especially of skin. The chief chemical property, which is believed to underlie their toxic action, is an affinity for -SH groups. Thus lewisite reacts with glutathione and thereby inactivates this vital substance. Lewisite is also a powerful inhibitor of pyruvic oxidase, the enzyme system catalysing the oxidation of pyruvate (Fig. 6). The toxic effects of lewisite on the animal, and the inhibition of pyruvic oxidase could both be prevented by di-thiol antidotes such as dimercaptopropanol (BAL), but not by monothiols (i.e. compounds containing one -SH group). On the other hand, the reaction of lewisite with the monothiol glutathione could be prevented by other simple monothiols. These results suggested that the major toxic action of lewisite was due to its action on the pyruvic oxidase system and that the reactive component in this enzyme system was a dithiol. In fact, the di-thiol compound, lipoic acid is a coenzyme for the oxidative decarboxylation of pyruvate. Inactivation of this coenzyme is therefore likely to be the mechanism of the lethal action of lewisite. The other effects of these chemical warfare compounds, vesication and mitotic inhibition in dividing cells, are less plausibly explained.

DEFICIENCY STATES AND RESPIRATORY ENZYMES

(See also Sebrell and Harris, 1955)

Thiamine

Thiamine is a constituent of the vitamin B complex, deficiency of which in the diet leads to Beri-Beri. Brain tissue from pigeons fed on a thiamine-deficient diet shows a deficient power to oxidise pyruvate. This failure is not due to lack of the appropriate enzyme but to deficiency of thiamine pyrophosphate which is a coenzyme required for the decarboxylation of pyruvate, a reaction which is a necessary prelude to the formation of acetyl CoA (Fig. 6). Thus once again blockage of a single stage in the tricarboxylic acid cycle brings respiration and energy production to a stop. An interesting variant of thiamine deficiency is provided by a lethal disease of foxes known as Chastek paralysis. This condition is due to thiamine deficiency induced not by lack of thiamine in the diet but by the enzymic destruction of thiamine due to the presence of thiaminase in raw fish fed to the animals at certain times of the year (Krampitz and Woolley, 1944).

Nicotinic Acid

This substance is also a member of the vitamin B complex and deficiency in man results in pellagra. Nicotinic acid is a component of the pyridine nucleotides DPN and TPN which act as hydrogen carriers in cell respiration and glycolysis (Figs. 3 and 6), and which are essential for energy generation. Lack of nicotinic acid in the diet is followed by inadequate synthesis of these coenzymes, a fall in their tissue level and failure of the enzymic reactions for which they are required. The clinical syndrome of pellagra is due partly to this cause, partly to other poorly understood factors.

Riboflavin

This member of the vitamin B complex may be lacking in the diet in malnutrition and in pellagra. Riboflavin is a component of two respiratory coenzymes, flavine mononucleotide and flavine adenine dinucleotide. The former is required for the shunt pathway of glycolysis in the oxidation of glucose-6-phosphate to 6-phosphogluconic acid (Fig. 5). Flavine adenine dinucleotide is part of the flavoprotein molecule which acts as a carrier in electron transport in respiration. Experimental dietary deficiency of riboflavin is followed by a progressive decline in the tissue levels of these flavoproteins.

Pantothene

Lack of pantothene may produce disease in rats, if not in man. The substance is part of coenzyme A, one of the most vital components of respiration and biosynthesis (Fig. 6).

Copper

Deficiency of copper produces disease in cattle and sheep. Many enzymes require a metal cofactor (prosthetic group) for proper function and copper is probably needed in this fashion by the respiratory enzyme cytochrome oxidase. Decreased activity of this enzyme is characteristic of copper deficiency and may well be responsible for many of the clinical symptoms, and eventually for death of the animal (Gallagher *et. al.,* 1956).

INJURY TO NON-RESPIRATORY ENZYMES

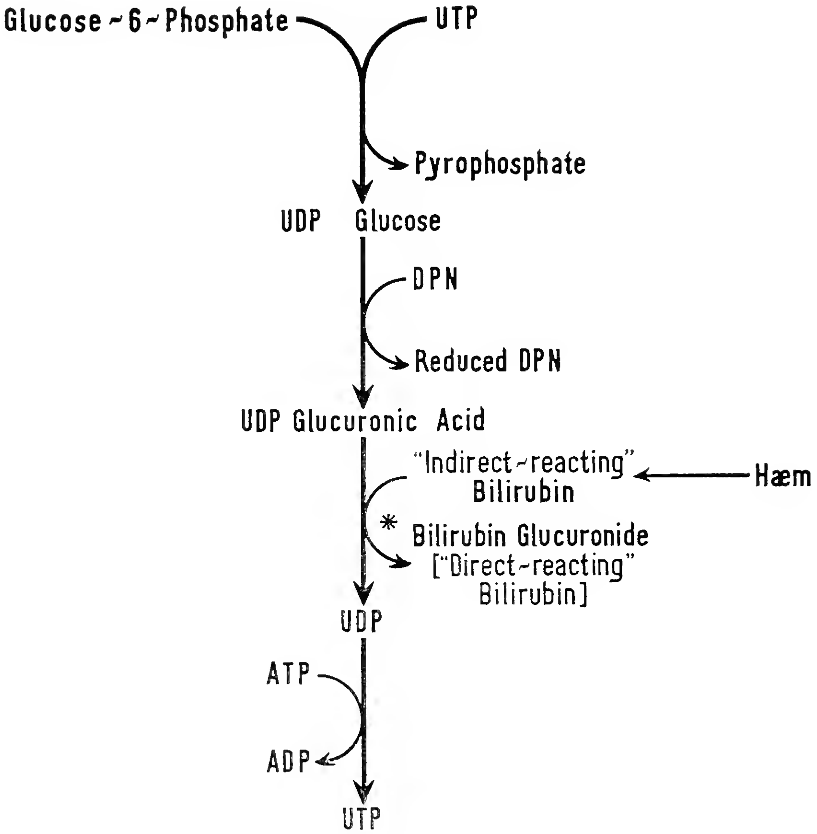
Non specific injury, e.g. the cutting off of blood supply to cells leads first to disorganisation of respiration and energy production and only secondarily to disruption of non-respiratory metabolism, e.g. biosynthesis and detoxication. However, special types of injury exist which damage specifically certain non-respiratory enzyme systems. Such injuries may be of endogenous origin or may de due to exogenous toxins, to deficiency states or to enzymic dysfunction of genetic origin.

ENDOGENOUS INJURY: KERNICTERUS (NUCLEAR JAUNDICE)

Kernicterus is a disease of the newborn in which there is bilirubinaemia and jaundice together with degeneration and bile-staining of parts of the brain, especially the nuclei of the basal ganglia. The condition is an interesting example of injury due to a temporary lack of enzymes controlling the detoxication of a potentially dangerous metabolite, namely indirect-reacting bilirubin (Billing and Lathe, 1958). When tested on isolated mitochondria, this substance brings oxidative phosphorylation to a standstill and in higher concentrations stops the oxidation of tricarboxylic acid cycle substrates.

Indirect-reacting bilirubin is formed from the haem liberated by the breakdown of red blood cells and is normally converted in the liver to the non-toxic direct-reacting (i.e. water soluble) pigment. This detoxication is accomplished enzymatically by conjugation with glucuronic acid in the presence of a coenzyme, uridine triphosphate (UTP). The process also requires ATP (Fig. 7). The newborn and especially the premature liver performs this enzymatic reaction inadequately thus accounting for the enhanced toxicity in the neonate of the many compounds normally detoxicated by this pathway. If the level of indirect-reacting bilirubin is especially high, due for example to massive haemolysis in blood group incompatibility such as Rh disease, large amounts of toxic pigment accumulate and damage particularly sensitive tissues, i.e. brain cells. The level of conjugating and detoxicating enzymes is very low in the newborn liver but rises steeply in the first few days of life.

Large doses of vitamin K are known to precipitate kernicterus in premature babies. This is thought to be due to interference with the supply of reduced glutathione, a coenzyme necessary for glycolysis in red cells and for the formation of haemoglobin from methaemoglobin. In the absence of reduced glutathione there is excessive haemolysis and a subsequent rise in circulating indirect-reacting bilirubin. Large doses of vitamin K are thought also to cause further damage to liver cells.


Glucose ~ 6 ~ Phosphate
UTP
Pyrophosphate
UDP Glucose
DPN
Reduced DPN
UDP Glucuronic Acid
"Indirect-reacting" Bilirubin
Hæm
*
Bilirubin Glucuronide ["Direct-reacting" Bilirubin]
UDP
ATP
ADP
UTP

* Enzyme catalysing this step is temporarily deficient in the neonatal liver and permanently absent in one variety of congenital jaundice.

Fig. 7. The Conversion of Bilirubin from the "Indirect" to the "Direct-Reacting" Form.

Diabetes

In diabetes mellitus carbohydrate cannot be fully utilised and supplies of oxaloacetate are thus reduced, this substance being formed chiefly by glycolysis. As a result, less citrate is formed, and the acetyl CoA that would normally condense with oxaloacetate is free to condense with other acetyl CoA to form acetoacetic acid which in turn forms acetone and β-hydroxybutyric acid, in other words, the ketone bodies excreted in the urine in severe diabetes (Fig. 8).

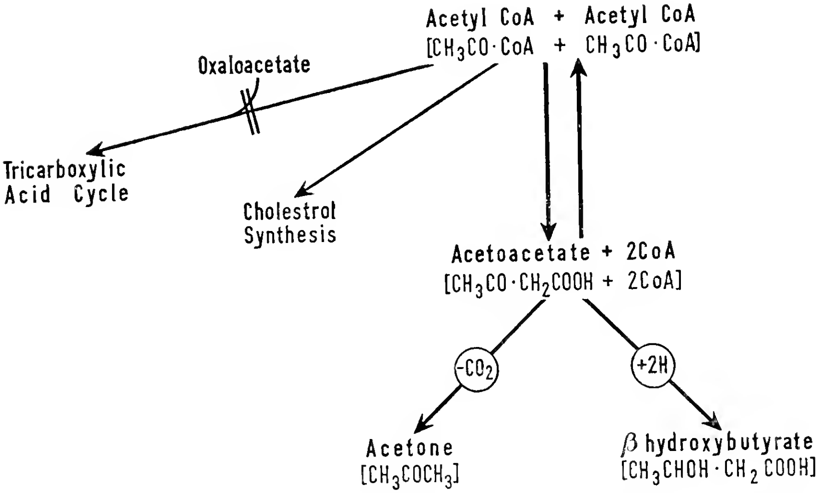

Acetyl CoA + Acetyl CoA
[CH3CO·CoA + CH3CO·CoA]
Oxaloacetate
Tricarboxylic Acid Cycle
Cholestrol Synthesis
Acetoacetate + 2CoA
[CH3CO·CH2COOH + 2CoA]
-CO2
+2H
Acetone
[CH3COCH3]
β hydroxybutyrate
[CH3CHOH·CH2COOH]

Fig. 8. The Formation of Ketone Bodies and Excess Cholesterol in Diabetes.

Another possible result of the disturbance is that acetyl CoA is diverted to another reaction in which it is involved, namely, cholesterol synthesis, thus leading to hypercholesterolaemia and perhaps to its deposition in blood vessels (atheroma). Both these abnormalities are common complications of diabetes.

EXOGENOUS POISONS

Well documented examples in this group are scarce. The anticholinesterases, e.g. DFP, parathion, mipafox, apiol produce severe neurological symptoms accompanied by demyelination and death. Their only major common property appears to be inhibition of certain carbonic esterases notably cholinesterase, and esterase linked proteases, e.g. chymotrypsin, but the connection between their effect on these enzymes and their toxic properties is uncertain.

The opposite effect, that of a specialised enzyme producing injury by virtue of its peculiar properties, may possibly be seen in the action of some bacterial toxins. Thus the α toxin of *Clostridium welchii* is a lecithinase which attacks phospholipids, important constituents of cell walls. This toxin in fact haemolyses red cells and disrupts isolated mitochondria.

DEFICIENCY STATES

Folic acid and cyanocobalamin (vitamin B_{12}) and its constituent cobalt may be deficient in man and animals and this lack may lead to anaemia and neurological disease. The two substances appear to be involved with the synthesis and interconversion of nucleic acids. Vitamin B_{12} is also involved in the transmethylation of methionine. The connection between these actions and the clinical results of their deficiency is unknown. Similarly, ascorbic acid (vitamin C) apart from aiding the metabolism of tyrosine, may be necessary to control the redox potential of cells. However, the link between these enzymic effects and scurvy (clinical vitamin C deficiency) remains tenuous.

GENETICALLY DETERMINED ENZYME DEFICIENCIES

(See also Harris, 1959)

A. Red Corpuscles

Sickle cell anaemia is a hereditary disease in which the red cells assume a characteristic sickle cell form, especially when subjected to low oxygen tensions. The condition appears to be due to an abnormality in the synthesis of the globin constituent of haemoglobin which is then present in a form that differs from normal in its electrophoretic pattern. This abnormal haemoglobin has the property of crystallising at low oxygen tensions, a feature which explains the distortion and framentation of red cells seen in sickle cell anaemia. In severe cases, this effect is seen at the oxygen tension of venous blood so that sickling is present continuously.

Another example is congenital methaemoglobinaemia. Here there is inability to reconvert methaemoglobin (normally formed continuously in small amounts) to haemoglobin. The disease is apparently due to absence of the flavoprotein enzyme in red cells that normally reduces methaemoglobin with the aid of reduced DPN as a hydrogen donor.

A somewhat more complicated picture is presented by Favism. Here there is a genetically determined sensitivity to the haemolytic action of the Fava bean. The disease is apparently due to a congenital absence from the red cell of the enzyme glucose-6-phosphate-dehydrogenase which is part of the "shunt" pathway of glycolysis (See Fig. 5). The activity of this enzyme is a major source of reduced TPN and absence of the enzyme leads therefore to deficient production of reduced TPN, which is associated in turn with a decline in the concentration of reduced glutathione in the red cell due to lack of available hydrogen ions. A fall in the level of this last substance is associated with abnormal sensitivity to haemolysis. Two possible explanations of this effect are that reduced glutathione, apart from being a general protector of enzymes susceptible to inactivation by oxidation, is required in glycolysis, being a coenzyme for triosephosphate dehydrogenase and hence for the synthesis of ATP, the chief energy source for the exclusion of water and sodium by the red cell. Abnormal entry of water and sodium is of course attended by haemolysis. Familial sensitivity to primaquine and naphthalene has a similar basis to Favism. In both cases glucose-6-phosphate-dehydrogenase is absent from the red cells although not from other tissues. The detailed mechanism of Favism and similar conditions is not altogether clear. It seems likely, however, that reduced glutathione protects against the haemolytic properties of certain agents and that its deficiency (due to absence of glucose-6-phosphate-dehydrogenase) is a central feature of this group of diseases.

B. Genetically Determined Enzyme Deficiencies in the Synthesis of Hormones

Goitrous cretinism is a congenital form of hypothyroidism usually associated with enlargement of the thyroid gland. It is due to a genetically determined failure to synthesise thyroid hormone following absence of one of at least three enzymes. The deficiency may be in the oxidase that converts inorganic iodide to iodine; in the dehalogenase that removes iodine from iodotyrosine or in the enzyme that couples iodotyrosine molecules to form thyroxin.

In the adrenal gland genetically determined absence of an enzyme involved in the biosynthesis of hydrocortisone may lead to virilism or hypertension due to a secondary overproduction of pituitary corticotropin. The failure may be in the hydroxylation of the steroid ring at the 21 or 11 carbon atom.

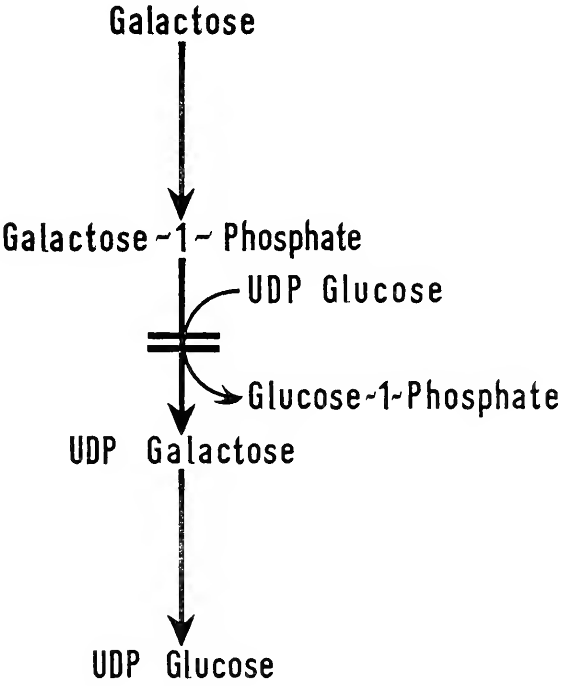


Fig. 9. The Mechanism of Galactosaemia.

C. Carbohydrate Metabolism

One example of a genetic abnormality in carbohydrate metabolism is galactosaemia. In this condition children are born with an inability to metabolise galactose (a constituent of lactose or milk sugar) which is in consequence excreted in large amounts. The abnormality is due to an inborn absence of the enzyme that couples galactose-1-phosphate to glucose-uridine di-phosphate (UDP). This complex would normally break down to UDP-galactose and glucose. As a result, less glucose than normal is made available and there is loss of calories and ATP. The clinical features of the disease are due to these effects and also to toxic effects of the accumulated galactose.

D. Amino Acid Metabolism

A well known example of a genetic enzyme inadequacy is phenylketonuria. Here there is absence of a liver enzyme that in the presence of TPN and oxygen converts phenylanine to tyrosine. The accumulated phenylalanine breaks down to toxic products, that damage the nervous system, mental deficiency forming a prominent feature of the clinical syndrome. Normal people fed large doses of phenylalanine do not produce these toxic products (phenylpyruvic, phenylacetic and phenylactic acids and phenylacetylglutamine). Their formation may indicate a further genetic abnormality or be an adaptive mechanism developed in response to long-continued high tissue levels of phenylalanine. One suggested mechanism for the toxicity of phenylalanine derivatives is interference with the enzyme 5-hydroxytryptophane decarboxylase which is needed for the synthesis of 5-hydroxytryptamine, a possible transmittor of impulses in the brain. Patients with this disease also show deficient melanin production. This defect is due to inhibition by phenyl alanine of the enzyme tyrosinase that catalyses the first step in the production of melanin from tyrosine.

REFERENCES

Baldwin, E. (1957) : *Dynamic Aspects of Pathology*, 3rd ed. Cambridge University Press.

Billing, B. H. and Lathe, G. H. (1958) : *Amer. J. Med., 24*:111.

British Medical Bulletin (1954) : *Reactions to Injury*, vol. 10.

Dawkins, M. J. R., Judah, J. D. and Rees, K. R. (1959) : *J. Path. Bact., 77*:257.

Gallagher, C. H., Judah, J. D. and Rees, K. R. (1956) : *Proc. Roy. Soc. B., 145*:134, 195.

Harris, H. (1959) : *Human Biochemical Genetics*. Camb. Univ. Press.

Hunter, D. (1955) : *The Diseases of Occupation.* English Universities Press.
Krampitz, L. O. and Woolley, D. W. (1944) : *J. Biol. Chem., 152:*9.
Peters, Sir Rudolph (1951) : *Proc. Roy. Soc. B., 139:*134.
Sebrell, W. H. and Harris, R. S. (1955) : *The vitamins,* vols. 1–3. Academic Press.

PART II

THE CHEMISTRY OF SOME TYPES OF CELL INJURY

Chapter 6

CELL DAMAGE PRODUCED BY TOXIC AGENTS

As described in the chapter devoted to enzymes, modern work suggests that many poisons produce their harmful effects by disturbing the respiratory mechanism of cells. In this section, which is devoted to a more detailed consideration of the subject, we shall therefore first of all recapitulate the main stages in cellular respiration, attempt to localise them within the cell and then see how poisons destroy cells by interrupting this aspect of metabolism.

CELL RESPIRATION (SEE ALSO CHAPTER 5)

Cell respiration makes use of the following devices:

1. The glycolytic system.
2. The hexose monophosphate shunt.
3. Enzymes of the Krebs cycle and of related aerobic metabolism.
4. The electron transport system.
5. The accompanying system for oxidative phosphorylation.

Many of these enzyme systems are concentrated in the mitochondria but other cellular components are certainly involved in some, if not all of these processes.

Glycolytic activity is a function of all mitochondria (de Buy and Hesselbach, 1956) but its exact localisation in the cell is still in doubt. Technical difficulties concerned in establishing this association are still considerable and it is uncertain whether the enzymes of glycolysis leak out from mitochondria during the manipulations used in the isolation of cellular components for such studies. During glycolysis glucose is broken down step-wise, by enzymic action and interactions with ATP, to pyruvate (See Fig. 4). Eventually the latter is transformed through aerobic metabolism to CO_2 and water with liberation of some of its latent energy for use by the cell.

An alternative pathway for glucose metabolism is available through the *hexose monophosphate shunt*. This is present for certain in the cells of the liver, mammary gland and adipose tissue (Abraham *et al.*, 1954). Glucose-6-phosphate is oxidised to phosphogluconic acid coupled with the reduction of the pyridine nucleotide, TPN. The acid is then decarboxylated to give pentose sugars which are broken down to CO_2 and water by way of the triose sugars (See Fig. 5).

Aerobic metabolism is largely concentrated within the mitochondria which are the chief site of the tricarboxylic (Krebs) cycle (Fig. 6). Isolated mitochondria, for instance, can catalyse the oxidation of a number of Krebs cycle intermediates and of fatty acids (Kennedy and Lehninger, 1949). The Ochoa system that couples phosphorylation with oxidation is also present in the mitochondria while succinic dehydrogenase and fumarase are generally accepted as enzymes exclusive to mitochondria (Schneider, 1959). Glutamic dehydrogenase which oxidises glutamic acid to α-ketoglutaric acid occurs exclusively in the mitochondria. Other oxidations placed there include those of fatty acids and choline, through betaine aldehyde to betaine. The distribution of some of the other enzymes of the cycle in the cell is still imperfectly known.

Electron carriers are involved in the secondary phases of oxidation (Fig. 3). Cytochrome oxidase is the only one of the substances so concerned that is known to be present exclusively in the mitochondria. About 10 per cent of the total cell diphosphopyridine nucleotide (DPN) is found in these organelles either firmly bound within them or attached to their membranes (Glock and McLean, 1955; Jacobson and Kaplan, 1957). The DPNH- and TPNH-cytochrome *c* phosphatases are also located in the mitochondria, but large amounts are found within the microsome fraction. Cytochrome *c* is largely associated with the mitochondria and cytochrome *b* and *d* have been detected there.

THE ANTI-METABOLIC ACTION OF SOME TOXIC SUBSTANCES

A wide variety of cytotoxic agents have now been studied and many of these exert their effects by damaging respiratory and biosynthetic mechanisms in the cell. These include such diverse substances as chlorpromazine, carbon tetrachloride, tri-ethyl-tin sulphate, bacterial toxins, thyroxin, heliotrine alkaloids and dimethylnitrosamine. However, we must be cautious in our statements since this is a field of investigation that is going on actively all the time and the conclusions may need revision as new facts are discovered.

Carbon Tetrachloride

Carbon tetrachloride has long been known to damage and kill the cells of the liver, kidneys and to a lesser extent of the heart muscle and brain when administered to animals and man by various routes. Most attention has been centered upon the liver where those effects are very vivid. Within twelve to twenty-four hours of giving a single toxic dose of carbon tetrachloride many of the liver cells congregated around the centrilobular veins develop large vacuoles, undergo fatty degeneration and die. This is followed by rapid mitotic proliferation of surviving cells and recovery in four to five days in the case of small animals. Clinical experience has made us familiar with similar disturbances of the liver in man but the renal cells are just as often or even more seriously damaged (Cameron and Karunaratne, 1936). Cytological changes are seen even within an hour or less of exposure to the poison. These show up as loss of the cytoplasmic basophilic bodies and glycogen with accumulation of lipids, but they are most likely a non-specific reaction of the cell to the poison for they are induced by a host of agents and even occur when the animal is starved of protein.

For some time now, pathologists have suspected that the mitochondria might be a major site of CCl_4 damage. The first convincing proof of this impression came when Christie and Judah (1954) isolated from homogenates of rat's liver poisoned by CCl_4 the typical "vacuoles" and showed them to be distorted, swollen mitochondria. Christie and Judah also showed that the oxidation of a number of Krebs cycle intermediates and of octanoate, pyruvate and glutamate by liver mitochondria was inhibited ten to fifteen hours after giving rats CCl_4. This effect could always be reversed except in the case of octanoate, by the addition of DPN. Similar upsets could be demonstrated *in vitro*. This suggested that CCl_4

might exert some physical action on the mitochondrial membrane rendering it more permeable to co-factors, especially DPN, and thus leading to failure of respiration and cell death. About the same time, Dianzani (1954) found that CCl_4 uncouples oxidative phosphorylation, increases the ATP-ase activity, induces loss of pyridine nucleotides and cytochrome *c* and diminishes the ATP content of the liver. He suggested that CCl_4 and other hepatotoxins destroy mitochondrial function and so deprive the cell of ATP and that this leads to a secondary failure of fatty acid activation. Dianzani recorded biochemical disturbances before fat accumulates in the liver and attributed the increase in fat to failure of fatty acid oxidation (1957). His observations were made on livers twenty-four hours or more after exposure to carbon tetrachloride, at a time when structural damage is pretty severe.

Recently more attention has been given to the fatty change that characterises liver cells in the early stages of CCl_4 intoxication. *In vivo* and *in vitro* studies show that CCl_4 reduces the 2:4 dinitrophenol-activated ATP-ase system and activates the Mg-ATP-ase-system; these events unfold side by side with loss of α-ketoglutarate and glutamic oxidase activity (Recknagel and Anthony, 1959). Such results suggest some mitochondrial disorganisation. But the total liver fat level is twice normal long before the mitochondria are apparently damaged which means that the cellular fat increases independently of impaired mitochondrial function. Moreover, in adrenalectomised animals twenty hours must elapse before the fatty change develops after exposure to CCl_4 and mitochondrial function is still normal. At this time the concentration of CCl_4 in the liver is low (Recknagel, Stadler and Litteria 1958).

Finally, protection of mitochondria against the action of CCl_4 is given by a variety of compounds, e.g. sulphaguanidine and phenergan; both protected and unprotected livers lose their cytoplasmic basophilia and glycogen, and accumulate fat, but the mitochondria remain unchanged in the protected liver (Rees, Spector and Sinha, 1961). Hence it seems likely that CCl_4 induces two separate changes in the liver cells; (1) fatty degeneration and (2) mitochondrial damage.

There seems to be little doubt that CCl_4 affects the integrity of the mitochondrial membrane, as originally suggested by Christie and Judah, but the mechanism for this still eludes us. Several workers have remarked upon the similarity of action of CCl_4 with that of inorganic phosphate which also makes mitochondria swell up, lose DPN *in vitro* and acquire water (Recknagel and Malamed, 1958). Ca ions would appear to be of importance in these responses. A ten-fold increase in concentration of Ca ions occurs in the mitochondria sixteen to twenty hours after exposure to CCl_4 (Thiers and Reynolds, 1958). Calcium ions release DPN from mitochondria *in vitro* (Ernster, 1956). It is possible, therefore, that the increased concentration of Ca ions in the liver in CCl_4 poisoning is the cause of the mitochondrial change. This may be due to these ions interfering with the binding of reduced pyridine nucleotides to mitochondria (Chance and Conrad, 1958). However, the loss of DPN from mitochondria exposed to CCl_4 *in vitro* cannot be referred wholly to calcium, for it happens when mitochondria are suspended in a calcium-free medium. Moreover, mitochondrial damage can be diminished by the administration of ethylene diaminotetroacetate (versene) which inhibits the Ca ion accumulation but does not prevent the fatty change (Calvert and Brody, 1958). It is difficult to escape from the feeling that many of these *in vitro* abnormalities of mitochondria isolated from rats poisoned by CCl_4 are artefacts due to calcium ions concentrating in the organelles. Such experiments may not represent accurately the behaviour of mitochondria in the living cell. Nevertheless, mitochondrial injury does occur since liver enzymes that are localised specifically in the mitochondria leak into the blood during CCl_4 poisoning though this happens at a relatively late period (Rees and Sinha, 1961). Perhaps CCl_4—a fat solvent—disturbs the lipoprotein of the mitochondrial membranes that occupy an essential place in the enzymic mechanism of these organelles (Green, 1959).

The story has taken a new turn as the result of recent investigations in our laboratory by Judah and Rees (1959). Reference to the chapter on Fats will remind the reader of the essential stages of lipid synthesis in the cell and recall the importance of choline in diverting diglyceride, through the action of transferase, in the proper direction of lecithin (Fig. 1). When this diversion is prevented through deficiency of choline, neutral fat is produced in excess and this accumulates within the cell because it is not so easily metabolised as lecithin. Judah and Rees showed that CCl_4 induces excessive phosphatidic acid production in liver cells so that even optimal supplies of choline are insufficient for the synthesis of lecithin and much of the diglyceride is diverted to neutral fat. This abnormality of metabolism sets in at an early stage of CCl_4 intoxication long before the mitochondria are damaged. Poisoning with carbon tetrachloride may thus make the cell relatively deficient in choline; the fatty degeneration that results could well be the outcome of a lack of lipotropic factor.

The necrosis of carbon tetrachloride poisoning is also being studied in our laboratory. The anti-histamine drug, Phenergan (promethazine HCl), greatly lessens the severity of the mitochondrial damage and necrosis, but leaves the fatty change unchecked (Rees, Spector and Sinha, 1961). It seems that fatty degeneration is a manifestation quite apart from mitochondrial damage, necrosis and the associated escape of enzymes from these cells. Adrenalectomy acts similarly to Phenergan. These findings suggest that both necrosis and mitochondrial damage are the outcome of a fundamental alteration of cell permeability which may even be secondary to disruption of normal electrolyte movements.

Thioacetamide

Thioacetamide has achieved some importance as a fungicide for the prevention of decay in oranges. In high doses it induces centrolobular necrosis in the liver of rats (Gupta, 1956). Workers in our laboratory have shown that it inhibits the respiratory metabolism of liver cells and their mitochondria, an effect which is associated with a high concentration of calcium ions in those cells (Gallagher *et al.*, 1956). If the Ca ions are inactivated by the addition of versene, respiration of liver homogenates and mitochondria will go on normally. Since calcium is a potent inhibitor of respiration and an uncoupler of oxidative phosphorylation *in vitro,* the inhibition of respiration in thioacetamide poisoning may be secondary to accumulation of calcium in the cell.

A further interesting disturbance can be traced to thioacetamide. Along with the calcium accumulation there is an increase of intracellular sodium and a decrease in potassium and magnesium. These ionic changes may well be the outcome of loss of semipermeability of the cell wall referable to an action there of thioacetamide and this idea of Gallagher *et al.* is certainly supported by their demonstration that the antihistamine, Phenergan, gives considerable protection against thioacetamide necrosis. This phenomenon, however, has no apparent connection with histamine release for thioacetamide itself does not release histamine from cells. The recent investigations of Judah (1960) suggest that the antihistamines inhibit some fundamental mechanism governing water and electrolyte movements by which mitochondria both swell and contract.

Heliotrine

Alkaloids with a pyrrolizidine ring occur in plants from the genera Senecio and Erectites (Compositae), Crotolaria (Leguminosae) and Heliotropium (Boraginaceae). Four of these alkaloids, retrorsine, isatidine, lasiocarpine and heliotrine cause liver necrosis in animals (Selzer *et al.,* 1951; Schoental *et al.,* 1954; Bull *et al.,* 1958). Heliotrine inhibits the DPN-dependent respiratory enzymes in liver cells and mitochondria. This occurs just when liver cell necrosis is becoming extensive, sometimes as early as one hour after exposure to heliotrine. Miss Margot J. Bailie (Thesis, 1959), working with Christie in E. J. King's laboratory at Melbourne, Australia, has shown that heliotrine picks out oxidative metabolism rather than the phosphorylation system while liver cells are necrosing. Only at a later stage, when necrosis is widespread, and DPN is partly successful in re-activating DPN-linked oxidation, is uncoupling of phosphorylation evident.

Dimethylnitrosamine

This compound destroys the centrolobular liver cells when given to laboratory animals by various routes (Barnes and Magee, 1954). It is quickly metabolised in the liver both *in vitro* and *in vivo* (Magee and Vandehar, 1958). During the early stages of poisoning the incorporation of C^{14} labelled amino acids into rat liver proteins is greatly reduced (Magee and Vandehar, 1958) so that failure to maintain the proteins of the cell in general, or to synthesise one or more specific protein involved in cell metabolism may be the cause of the "biochemical lesion" that ultimately leads to cell death. According to Miss Margot J. Bailie (Thesis, 1959), dimethylnitrosamine causes leakage of DPN from mitochondria so that the cell loses its stores of this compound. Such an action recalls the "ageing" of mitochondria whereby they no longer concentrate certain ions after storage at low temperature. "Aged" mitochondria sustain a loss of nucleotides, including DPN, and accumulate water (Ernster and Lindberg, 1958). At the same time, DPN-linked oxidations and oxidative phosphorylation are inhibited or lost. More recent work by Farber and Magee, as yet unpublished, suggests an alternative mode of action for the action of this substance on liver cells. Thus the injection of dimethylnitrosamine (DMN) into rats is followed by the appearance of 7-methylguanine in the liver ribonucleic acid (RNA). Since this purine base is not known to occur in normal rat liver RNA its presence suggests that methylation of RNA has taken place. Earlier evidence of several kinds has suggested that DMN must be activated metabolically before it can damage cells, and diazomethane has been suggested as a possible toxic metabolite. Diazomethane is a powerful methylating (i.e. alkylating) agent, highly toxic and a known mutagen. It has been shown to methylate guanosine preferentially in the seven position in the test tube. Hence it is suggested that the liver necrosis is caused by methylation of cell constituents, including RNA and protein, and that the later occurring liver cancer may be related to the presence of RNA containing the abnormal purine base, 7-methyl guanine.

Chlorpromazine

The "tranquilizer" chlorpromazine inhibits cytochrome oxidase and phosphorylase systems in the rat's brain (Abood, 1955). In liver cells *in vitro* it interferes highly selectively with one or two of the phosphorylation steps during oxidative phosphorylation (Dawkins, Judah & Rees, 1959). Chlorpromazine produces no definite structural damage in cells though it leads to jaundice associated with stasis of bile flow within the minute bile passages of the liver.

Triethyl Tin Compounds

Alkyl tin compounds are used a great deal in the plastics industry and have been suggested as fungicides. Most tin compounds are insoluble in the body fluids and so are harmless. Many of the alkyl derivatives are soluble and toxic, especially triethyl compounds. The triethyl derivatives give paresis, tremors and convulsions in animals and induce a striking interstitial oedema of the white matter of the brain and spinal cord of the rat without obviously damaging the neurones. *In vitro* they interfere with oxidative phosphorylation by mitochondria (Aldridge and Cremer, 1955) but the relationship of this finding to the *in vivo* effects is far from certain. The toxic action of triethyl tin compounds can be forestalled by premedication with dimercaprol (BAL) which suggests that they may exert their effect by inhibiting a thio-enzyme (see also Lewisite).

Bacterial Toxins

Diphtheria toxin has so far defied all attempts to discover the nature of its biochemical lesion. When given in large doses to susceptible animals a long latent period of many hours intervenes in which no morphological or biochemical disturbances can be demonstrated. Such arresting features of intoxication as increased resistance of the animal to insulin, reduced capacity to synthesise carbohydrates or to metabolise lactic acid, (Popjak, 1948) with decrease in the stores of muscle phosphocreatine and increased water content of cells are all secondary effects that appear many hours after a primary injury that baffles the investigator (Holmes, 1939). Perhaps there is competitive inhibition or block of synthesis of the cytochromes in the diphtheritic animal. The succinoxidase system appears to be susceptible to diphtheria toxin (Pappenheimer and Williams, 1952). Recent studies with stabilised growing HeLa cells show that the toxin is rapidly adsorbed and quickly inhibits protein synthesis. Anaerobic respiration or glycolysis remains unaffected but some inhibition of succinoxidase is induced (Strauss and Hendee, 1959).

Clostridium welchii toxins produce severe necrosis of muscle with liberation of gas. They constitute a group of toxic proteins, the chief one of which, α toxin, is a very active lecithinase that splits the phospholipid lecithin in the cell wall and mitochondrial membranes to yield phosphoryl choline and the diglyceride. In this way the cells and mitochondria are disrupted at least *in vitro* (Payling Wright, 1955).

Botulinum toxin, perhaps the most toxic of all known poisons, produces no specific structural disturbance in the cell, but it injures the terminal, largely unmyelinated portion of cholinergic nerves whether they are pre- or post-ganglionic components of the autonomic nervous system or somatic nerves supplying skeletal muscle. So far no biochemical lesion has been spotted in affected cells. The clinical features are satisfactorily explained on the basis of some lesion produced by the toxin at the myoneural junction which renders that structure incapable of releasing acetylcholine in quantities sufficient to evoke a contraction of the muscle fibre (Payling Wright, 1955).

Tetanus toxin, it is generally agreed, operates at some site within the central nervous system to give widespread skeletal muscle spasticity and convulsions. No characteristic cellular changes have been discovered and its mode of action is uncertain. On the whole it appears to behave "less as a general neural poison than as a specific impediment to acetylcholine formation and release—a property which it shares with botulinum toxin" (Payling Wright, 1955). Tetanus toxin, however, selects central cholinergic mechanisms as its main site of action, botulinum toxin chooses peripheral mechanisms. Tetanus toxin also impairs reflex inhibition in the spinal cord. The biochemical lesions responsible for these disturbances are not known.

Typhimurium toxin gives cloudy swelling and focal necrosis in the liver of rats. Mitochondria from affected liver cells show uncoupling of oxidative phosphorylation *in vitro*, a biochemical lesion possibly responsible for the appearance of cloudy swelling, if not for necrosis (Fonnescu and Severi, 1956).

REFERENCES

Abood, L. G. (1955): *Proc. Soc. exper. Biol. Med., 88*:688.
Aldridge, W. N. and Cremer, J. E. (1955): *Biochem. J., 61*:406.

Barnes, J. M. and Magee, P. N. (1954) : *Brit. J. Indust. Med., 11*:167.

Bull, L. B., Dick, A. T. and McKenzie, J. S. (1958) : *J. Path. Bact., 75*:17.

Buy, A. G. de and Hesselbach, M. L. (1956) : *J. Histochem. Cytochem., 4*:36.

Buy, A. G. de and Hesselbach, M. L. (1958) : *J. Nat. Cancer Inst., 20*: 403.

Calvert, D. N. and Brody, T. M. (1958) : *J. Pharm. Exp. Therap., 123*: 273.

Cameron, G. R. and Karunaratne, W. A. E. (1936) : *J. Path. Bact., 42*:1.

Chance, B. and Conrad, H. (1958) : *Fed. Proc., 17*:200.

Christie, G. and Judah, J. D. (1954) : *Proc. Roy. Soc. B., 142*:241.

Dawkins, M. J. R., Judah, J. D. and Rees, K. R. (1959) : *Biochem. J., 72*: 204.

Dianzani, M. U. (1954) : *Biochim. Biophysic. Acta, 14*:514.

Dianzani, M. U. (1957) : *Biochem. J., 65*:116.

Ernster, L. (1956) : *Exper. Cell Res., 10*:704.

Ernster, L. and Lindberg, D. (1958) : *Ann. Rev. Physiol., 20*:13.

Fonnescu, A. and Severi, C. (1956) : *J. biophys. biochem. Cytol., 2*:293.

Gallagher, C. H., Gupta, D. N., Judah, J. D. and Rees, K. R. (1956) : *J. Path. Bact., 72*:193.

Glock, D. E. and McLean, P. (1955) : *Biochem. J., 61*:388.

Green, D. E. (1959) : *Advances in Enzymol., 21*:73.

Gupta, D. N. (1956) : *J. Path. Bact., 72*:183.

Holmes, E. (1939) : *Physiol. Rev., 19*:439.

Jacobson, K. B. and Kaplan, N. O. (1957) : *J. biol. Chem., 226*:603.

Judah, J. D. (1960) : *Nature, 185*:390.

Judah, J. D. and Rees, K. R. (1959) : *Fed. Proc., 18*:1013.

Magee, P. N. and Vandehar, M. (1958) : *Biochem. J., 70*:600.

Pappenheimer, Jr., A. M. and Williams, C. M. (1952) : *J. gen. Physiol., 35*:727.

Popjak, G. (1948) : *J. Path. Bact., 60*:75.

Recknagel, R. O. and Anthony, D. D. (1959) : *J. biol. Chem., 234*:1052.

Recknagel, R. O., Stadler, J. and Litteria, M. (1958) : *Fed. Proc., 17*:129.

Recknagel, R. O. and Malamed, S. (1958) : *J. biol. Chem., 232*:705.

Rees, K. R. and Sinha, K. P. (1960) : *J. Path. Bact., 80*:297.

Rees, K. R., Spector, W. G. and Sinha, K. P. (1961) : *J. Path. Bact. 81*: 10.

Schneider, W. C. (1959) : *Advances in Enzymol., 21*:1.

Schoental, R., Head, M. A. and Peacock, P. R. (1954) : *Brit. J. Cancer, 8*:458.

Selzer, G., Parker, R. G. F. and Sapeika, N. (1951) : *Brit. J. exper. Path., 32:*14.

Strauss, N. and Hendee, E. D. (1959) : *J. exper. Med., 109:*145.

Thiers, R. E. and Reynolds, E. S. (1958) : *Fed. Proc., 17:*537.

Wright, G. Payling (1955) : *Pharmacol. Rev., 7:*413.

Chapter 7

DIETARY LIVER NECROSIS

THE LAST TWENTY-FIVE years has seen the emergence of a new chapter in the history of nutritional defects that offers important lessons for cellular pathology. Two distinct types of disturbance of the liver have now been traced to dietary deficiency; it is one of these we wish to discuss in this section. The other type, in which the liver becomes fatty and in time cirrhotic, is caused by a lack of choline, betaine or methyl precursors, vitamin B_{12} or folic acid. We have already dealt with this topic in the chapter on fats. The condition that we are about to discuss is called "dietary liver necrosis" or "acute massive necrosis of the liver."

THE DISCOVERY OF DIETARY LIVER NECROSIS

In 1935, Weichselbaum, working at Edinburgh, noticed that rats fed diets low in protein and deficient in l-cystine (and quite likely methionine) became ill and died after approximately six weeks. The liver of such animals was swollen and haemorrhagic, and presumably necrotic, but Weichselbaum gave no histological description from which to decide this point. Definite liver changes were noted by György and Goldblatt (1939) who fed rats on low casein diets deficient both in lipotropic factors and in thio-amino acids. They showed that casein and methionine could protect rats. Meanwhile, Himsworth and Glynn (1945) had formed very similar opinions and gave the first detailed account of massive dietary necrosis in the liver. They also showed that bacterial or viral infection played no vital part in the process. Similar results were obtained by Daft, Sebrell and Lillie (1942), who found that the liver damage could be prevented by adequate supplies of methionine and cystine. Since choline had no influence on the necrosis it seemed likely that this was a distinct entity from fatty liver and cirrhosis. German investigators likewise had encountered these effects of cystine deficiency (Hoch and Fink, 1943). As a result of these investigations the importance of thio-amino acids seemed fairly established. However, Schwarz (1944) announced that diets had to be simultaneously deficient in both thio-amino acids and in α tocopherol to be effective in inducing massive liver necrosis. Meanwhile an interesting controversy went on between American and British workers about the part played by yeast, the composition of which varies considerably in the two countries. From this dispute came the discovery by Schwarz (1951) in ordinary casein and most American yeasts but not in most European yeasts of factor 3, a substance that protected livers against dietary necrosis. It was then found that factor 3 relies largely on its selenium content for its activity (Schwarz and Foltz, 1957). Cystine likewise may owe its protective property to contamination with selenium and not to any property of the amino acid molecule. Hence dietary liver necrosis develops when there is a simultaneous lack of α tocopherol (vitamin E) and an organic compound of selenium (factor 3) (Schwarz *et al.,* 1959). The presence of inorganic selenium in the diet is usually sufficient to protect against the necrogenic effects of this deficiency. The necrosis, however, is influenced by other factors such as food intake, environmental temperature, age of the animal, endocrine status and level of dietary fat apart from α tocopherol. It is possible also that the intestinal bacteria may play a part in the initiation of dietary liver necrosis (Gyorgy, Stokes, Goldblatt and Popper, 1951; Beveridge, 1954).

THE STRUCTURAL CHANGES OF DIETARY NECROSIS

Dietary liver necrosis takes some time to develop, according to the nature of the deficiency. In some instances rats have shown necrosis after two to three weeks of feeding, in others necrosis takes forty-eight to eighty-eight days to develop. Hence there is a prenecrotic and necrotic phase. The latter very often kills quickly though sometimes animals survive only to pass into postnecrotic scarring of the liver some months later.

There are no characteristic cytological disturbances before necrosis appears. Shrinkage of liver cells from loss of cytoplasm, decrease of basophilic (RNA) granules and a steady increase of fat in the centrolobular cells, but no alteration in glycogen content, are constant, but their significance is uncertain since they are met with after any kind of protein deficiency (Kosterlitz, 1947).

Massive necrosis sets in explosively. The animal may be in perfect health up to six hours before death, and then suddenly is struck down. In the earliest stages, parenchymal degeneration and necrosis are midzonal and peripheral. Many contiguous liver lobules are soon destroyed while the central and middle zones of many other lobules are involved. Necrosis is coagulative to begin with, and is accompanied by severe nuclear degeneration, but there is little inflammatory reaction, no disturbance in the bile ducts or blood vessels. Later the liver cells break up into a pale, eosinophilic debris stippled with nuclear fragments, while glycogen is completely lost in necrotic cells and is much reduced in surviving cells around the portal tracts (Himsworth, 1950; Abell *et al.*, 1950). Calcification of cellular debris is not uncommon and fat heaps up within unaffected liver and Kupffer cells. Regeneration is prominent in surviving cells after a few days and may lead to the formation of regeneration nodules in the stage of post-necrotic scarring.

CHEMISTRY OF THE LIVER CELLS DURING DIETARY NECROSIS

Little is known about the chemical changes preceding and accompanying dietary necrosis. In the pre-necrotic period there is an initial and proportional fall in both dry and wet liver weights. Total lipids, neutral fat, total fatty acid and cholesterol esters show progressive increase, but no additional significant change is found until the onset of necrosis. Glycogen and phospholipid levels remain unchanged.

With massive necrosis water and plasma accumulate in the organ giving a considerable increase in wet and dry weights. Free cholesterol is increased, but phospholipid and glycogen greatly diminish in amount (Abell and Beveridge, 1950).

Liver slices from pre-necrotic animals consume oxygen at a normal rate for approximately thirty minutes, but the oxygen uptake declines to 30-50 per cent normal during the following hour (Chernick *et al.*, 1955). This metabolic defect is prevented by agents that prevent liver necrosis. Dying livers also show progressive failure of ketogenesis, lipogenesis and oxidation of acetate to CO_2 (Rosecan *et al.,* 1955) . Mitochondria isolated from the liver at this stage show impaired succinic dehydrogenase activity, presumably because of altered permeability especially when diphosphopyridine nucleotide is added to the medium (Corwin and Schwarz, 1959) .

Quite recently our colleague Dr. André McLean has made an important contribution to our knowledge of the pre-necrotic stage. He has studied the water and ion balance of liver slices incubated in Ringer solution. Slices from pre-necrotic liver are unable to maintain their internal potassium concentration. Slices from control animals, given vitamin E, show no such defect.

This defect is found long before the respiratory decline described by Schwarz's group. McLean suggests that respiratory decline is secondary to alteration of water and ion distribution in the liver slices. (See also Chapter I.)

The finding that selenium prevents dietary necrosis but has no effect on any of the biochemical lesions so far discovered suggests that we have not yet defined the biochemical lesion of dietary liver necrosis. However, the description of a lesion that affects primarily intracellular water and ion balance is of interest. Such lesions may well be found in other pathological states.

REFERENCES

Abell, M. R., Beveridge, J. M. R. and Fisher, J. H. (1950) : *Arch. Path., 50:*1.

Beveridge, J. M. R. (1954) : *Canad. Med. Assoc. J., 70:*267.

Corwin, L. M. and Schwarz, K. (1959) : *J. B. C., 234:*191.

Chernick, S. S., Moe, J. G., Rodrian, G. P. and Schwarz, K. (1955) : *J. B. C., 217:*829.

Daft, F. S., Sebrell, W. H. and Lillie, R. D. (1942) : *Proc. Soc. exp. Biol. Med. N. Y., 50:*1.

György, P. and Goldblatt, H. (1939) : *J. exper. Med., 70:*185.

György, P., Stokes, J., Goldblatt, H. and Popper, H. (1951) : *J. exp. Med., 93:*513.

Himsworth, H. P. (1950) : *Lectures on the Liver and Its Diseases,* 2nd ed. Oxford.

Hoch, A. and Fink, H. (1943) : *Z. physiol. Chem., 278:*138.

Himsworth, H. P. and Glynn, L. E. (1945) : *Clin. Sci., 5:*93.

Kosterlitz, H. W. (1947) : *J. Physiol., 106*:194.
McLean, A. (1960) : *Nature, 185*:936.
Rosecan, M., Rodman, G. P., Chernick, S. S. and Schwarz, K. (1955) : *J. biol. Chem., 217*:967.
Schwarz, K. (1944) : *Z. physiol. Chem., 281*:109.
Schwarz, K. (1951) : *Proc. Soc. exper. Biol. Med., 78*:852.
Schwarz, K. and Foltz, C. M. (1957) : *J. Amer. Chem. Soc., 79*:3292.
Schwarz, K., Stesney, J. A. and Foltz, C. M. (1959) : *Metabolism, 8*:88.
Weichselbaum, T. E. (1935) : *Quart. J. exper. Physiol., 25*:636.

Chapter 8

DAMAGE OF THE HEART MUSCLE CELL DUE TO ANOXIA

METABOLISM OF NORMAL CARDIAC MUSCLE

THREE STAGES ARE RECOGNISABLE in the metabolism of normal cardiac muscle. During the first of these the heart muscle abstracts fuel from the blood and metabolises it. The energy so liberated is largely saved from dissipation and diverted by special channels to the contractile mechanism where it is utilised in a series of complex reactions (Bing, 1956; Olson and Patnek, 1959) .

Energy Liberation

Cardiac fuel consists largely of free fatty acids, glucose, lactate and pyruvate which are extracted from the capillaries of the myocardium and so changed that their energy is set free. Acetate, ketone bodies and amino acids are also used, but to a much lesser extent. Indeed, the heart can obtain energy from every form of fuel available to it in the blood. Pyruvate seems to be its favourite but the muscle cells can shift from one preference to another. After a meal or during the infusion of sugar, glucose, lactate and pyruvate become the chief sources of energy, and consequently the cardiac respiratory quotient is nearly 1.0. Following overnight fasting, fatty acids are mainly utilised and the respiratory quotient drops to about 0.82. With prolonged fasting and in diabetes mellitus carbohydrates are even less utilised by the heart and its R.Q. falls as low as 0.70 showing that now it depends almost wholly on fatty acids for energy. When much food is available, therefore, carbohydrate provides most of the heart's energy but when fasting the organ depends largely upon fats.

The heart is well adapted for continuous aerobic work since it is three to four times as vascular as skeletal muscle and its blood flow is 10 to 20 times as great per unit weight of tissue. (Olson and Schwartz, 1951) . Moreover, the heart muscle cells are very good at extracting oxygen from the blood, and nowhere else in the body are the oxidative enzymes and coenzymes, e.g. cytochrome *c*, cytochrome oxidase and succinic dehydrogenase, so concentrated as in the heart. Finally, energy production and utilisation are more closely adjusted here than in other muscles which means that the myocardium does not need to carry big stores of creatine phosphate as a provision for reserve energy (Lipmann, 1941) nor does it run into oxygen debt when carrying a normal work load.

The fuels that we have mentioned are broken down to a common intermediate, acetyl-co-enzyme A; the acetyl group then condenses with oxalacetate to yield citrate, and the familiar tricarboxylic acid (Krebs) cycle (Fig. 6) is embarked upon to yield 8 H atoms or electrons ($H = H^+ + e$). These represent the energy content of the acetyl fragments and are equivalent to the 4 H atoms associated with the acetyl group together with 4 H atoms derived from water that is added to cycle intermediates in the course of the oxidation of the fragments. Each oxidative step of the cycle is catalysed by glycolytic, lipolytic and citric acid cycle enzymes, many of which reside within the numerous sarcosomes or mitochondria of the heart muscle cells. With each step, too, there is esterification of inorganic phosphate, leading to the creation of high energy phosphate bonds. In this way the bond energy of fuel brought to the myocardial cells is converted into electrons for transport to oxygen.

Energy Conservation

Energy set free during these various oxidative processes is conserved through the mechanism of oxidative phosphorylation which has already been considered in Chapter 5. There the business of electron flow along the H transport chain is described. In this way phosphorylation of adenosine diphosphate (ADP) is coupled to the oxidation of the cytochrome enzymes. Creatine phosphate stores, although relatively small in the heart, also provide some high energy phosphate capacity by virtue of the enzyme transphosphorylase. In either case, ATP is the great energy transmitter. The mitochondria are important sites for these processes. Cardiac mitochondria are less susceptible to inhibitors than are mitochondria from other organs, but when damaged they seriously disturb the functioning of the muscle cells.

Energy Utilisation

Adenosine triphosphate (ATP) carrying the high-energy phosphate bond is directed into the contractile mechanism of the muscle cell and there it gives up its energy for the performance of mechanical work. It is still not known for certain how this happens but the following account is generally accepted (Szent-Györgyi, 1953; Bailey, 1956).

At least two contractile proteins are present in muscle. They are known as actin and myosin. In the resting state, actin and a myosin-ATP complex exist independently in a dissociated form separated by the electro-positive ionic environment created by the surrounding K ions. Part of the myosin molecule (L-meromyosin) is thin and straight and is kept from folding by repulsive forces between K ions attracted by ionised groups in close proximity along the molecule. When a wave of excitation passes this equilibrium is disturbed. Actin then combines with the myosin-ATP complex to give the contractile protein actomyosin. The characteristics of the myosin molecule are changed and in the presence of ATP the thin L-meromyosin segment becomes more pliable. This segment collapses, the myosin molecule is shortened and the muscle cell contracts. ATP is converted to ADP and inorganic phosphate during this reaction. For relaxation, the heart requires ATP, creatine phosphate (CP) and Mg ions. A heat-stable protein, identified as myokinase, is also needed for the renewal of energy expended during contraction. Szent-Györgyi (1956) postulates in relaxation a dissociation of actin and myosin and the regeneration of ATP, presumably in part at least from transfer of high-energy phosphate from CP to ADP. But it is the intracellular ionic environment that determines the changes, by influencing the balance of repulsive and attractive forces, and the tendency of actin and myosin to part or to join. The ionic environment, in turn, is regulated by factors governing the permeability of the cell membranes. (The work efficiency of the phase may be as high as 60 per cent with an over-all work efficiency in the heart muscle of 35 to 40 per cent.)

It is thought that actin filaments arise from either end of the sarcomere or muscle unit, myosin filaments from the middle of the sarcomere, and they then interdigitate (Huxley, 1956). In the relaxed state filaments slide easily along each other. During contraction this sliding is brought about by internal forces, probably by obliquely orientated cross linkages between actin and myosin filaments. There is no actual shortening of myosin filaments until they are crowded together by extreme shortening of the sarcomeres. Szent-Györgyi describes a "plasticizing" effect of ATP. After a change in muscle length has occurred, either by stretching or shortening, the plasticizing quality allows the muscle to regain a tension about equal to that originally held. Without this effect a muscle becomes rigid and responds to slow stretch with an increase in tension, finally tearing if the stretch goes too far.

K ions are involved in some way, we have seen, in the work of the cardiac muscle. They are necessary for optimal work and maintenance of the stability of isolated heart muscle mitochondria. Muscle poor in K ions fatigues easily and develops less tension. During contraction K^+ leaves the muscle cell and Na^+ enters through the semi-permeable cell membrane (Hodgkin, 1951). At the end of this phase Na^+ is extruded actively and K ions than diffuse in, driven against the concentration gradient by the potential gradient, to re-establish an equilibrium with a relatively high concentration of K^+ and low concentration of Na^+ in the cell.

From these considerations it follows that myocardial metabolism can be upset through (1) disturbances in energy production, and (2) disturbances in energy utilisation. Instances of (1) are met with in the ischaemia of coronary occlusion, haemorrhagic shock and ventricular fibrillation. A special instance is heart failure associated with Beri Beri. Under group (2) comes cardiac failure.

Energy production can fail at any level of the catalysed processes concerned in its machinery. Since these are closely interrelated it is not surprising that one perverted phase of metabolism may lead to changes in other metabolic pathways, as in the case of diabetes mellitus. On the other hand, as with cardiac ischaemia, the disturbance may remain more localised. Interference with the cocarboxylase mechanism, as in haemorrhagic shock and coronary occlusion, blocks the incorporation of carbohydrate catabolism into the Krebs cycle. It further interrupts fatty-acid catabolism because the flavine co-factor required for the first step in fatty-acid catabolism cannot be reoxidised under anaerobic conditions. Hence energy production is seriously curtailed. But in diabetes mellitus, myocardial efficiency is not diminished because the devices for energy production are probably not so severely disturbed and alternative metabolic pathways are preserved, even though carbohydrate, fat and protein metabolism are impaired. In Beri Beri, deficiency of thiamine leads to depletion of tissue co-carboxylase and a block may intervene between pyruvate and acetyl co-enzyme A, thus preventing completion of carbohydrate metabolism through the citric acid cycle. Here blood concentrations of pyruvate and lactate increase and myocardial failure follows because interference with energy production is severe.

RESPONSE OF THE MYOCARDIAL CELL TO ISCHAEMIA

We are largely dependent upon animal experiments for our knowledge of what happens to the heart soon after its arterial supply is greatly diminished. Within *one hour* of such restriction the muscle fibre glycogen is greatly reduced, and a substance that stains with the P.A.S. method (and therefore presumably containing polysaccharide aldehyde groups) appears in the cells (Yokoyama *et al.*, 1955). Oxygen consumption declines rapidly, but returns to normal levels if coronary flow is restored *within 2 hours* of the initial obstruction. After ischaemia for *four to five hours* the myocardium completely loses its ability to extract oxygen from the available supply of blood. It can still extract glucose but pyruvate extraction falls off rapidly and lactate and glucose production greatly increase. These findings suggest that aerobic pathways of metabolism are now failing and that anaerobic mechanisms are intervening.

However, enzyme activity still remains unimpaired. At two hours after coronary occlusion DPN is considerably diminished, but there is no change in co-carboxylase, nor is the succinic acid dehydrogenase or cytochrome-cytochrome oxidase system affected. Lactic and pyruvic dehydrogenases are also conserved for some time. But ionic disturbances set in rapidly and the resting membrane potential can no longer be maintained because of failure of the mechanisms that maintain ionic gradients. Action potentials, although they cannot be generated and propagated, may disappear before the metabolic processes or the contractile proteins are irreversibly damaged. A decline in ATP has been recorded along with weakened contractile response to electrical stimulation and eventually rigor mortis supervenes in the anoxic heart muscle (Greiner, 1952). After death or in complete anoxia the pH of muscle cells falls with the production of lactic acid according to the glycogen stores available at the time of death. The turnover rate of ATP, too, is affected by change in H ion concentration. The pH dependence curve of ATP breakdown is very similar to the myosin-ATPase activity curve and suggests that the two may be related (Bendall, 1951).

Ischaemic changes in muscle proteins are, in all probability, similar to those that occur with rigor mortis. Actomyosin not only fails to dissociate and reverse muscle contraction, but abnormal cross linkages form between different filaments (Szent-Györgyi 1953). Huxley (1956), from low angle x-ray studies of muscle in rigor, suggests that the secondary filaments crystallise out permanently, thus rendering potentially reversible changes in the contractile proteins irreversible.

RESPONSE OF THE MYOCARDIAL CELL TO ANOXIA AND HAEMORRHAGIC SHOCK

When an organ demands oxygen the need may be met by (1) increasing the blood flow, or (2) increasing the amount of oxygen extracted from the available blood. The heart usually responds by the first method, but there is some evidence, at any rate in the dog's heart, that more oxygen may be extracted under such circumstances (Hackel *et al.*, 1954), although this is not significant until the increased demand becomes extreme.

As the oxygen supply fails and the muscle cell receives less than is necessary to maintain its normal aerobic metabolism the oxygen tension in the myocardium falls. This shifts the oxidation-reduction system towards a more reduced state. The extent to which individual members of the electron carrier system shift towards the reduced form will be determined by the inherent redox potential of the system. DPN has the lowest potential in the carrier system and the rates of oxidation for energy production will not be altered until DPN has been affected (Huckabee, 1958). When oxidative potential becomes low enough, the metabolic systems are involved. The first DPN complex system to be reversed will be the one with a potential closest to that of DPN: $DPNH_2$ or the lactic dehydrogenase system.

$$\text{Pyruvate} + \text{DPNH}_2 \overset{\text{(LDH)}}{\rightleftharpoons} \text{Lactate} + \text{DPN}$$

An increase of $DPNH_2$ would swing the equation to the right with the production of lactate and the oxidation to DPN. Because lactate does not take part in any other equilibrium, this reaction acts as a safety valve in the presence of anoxia thereby permitting other metabolic systems to go on functioning. Eventually anoxia leads to a decrease in myocardial extraction rate of lactate with serious results for the cell.

In *haemorrhagic shock,* the contractile power of the heart is reduced because the muscle cells have less oxygen at their disposal. This is brought about in two ways. (1) through reduced coronary blood flow and (2) by failure of the cells to extract more oxygen from the blood. As a result the heart expels less blood at each beat, stagnant anoxia sets in peripherally and the tissue cells so affected rob the blood of more and more oxygen. This in turn leaves less oxygen for the heart and the blood concentrations of glucose, lactate and pyruvate rise. The heart muscle, however, extracts more lactate, but less pyruvate and glucose, even when the lost blood is replaced by transfusion, which suggests that its metabolic impairment is initiated by loss of blood but is not corrected by such a transfusion. The increased extraction of lactate that we have noted in shock suggests that the enzymatic pathways through which lactate is directed are not interfered with, but the cause of this lactate extraction is not known. Co-carboxylase is destroyed under anaerobic conditions, possibly because of its dephosphorylation. Hence the myocardial block in haemorrhagic shock may be similar to that encountered in Beri Beri, although the latter is referable to an inadequate supply of thiamine in the food, the former to anoxia.

HEART FAILURE

In congestive cardiac failure due to *valvular disease* with decreased cardiac output, the coronary blood flow and myocardial oxygen usage are unchanged. Carbohydrate and fatty acid extraction are but slightly reduced and there is no alteration in oxidative metabolism. There is, however, some evidence of a biochemical defect in the contractile mechanism itself. The molecular weight of cardiac myosin for normal dogs is about 225,000, for dogs in valvular cardiac failure about 750,000. Probably some abnormality of myosin aggregation prevents the formation of an acto-myosin with normal contraction properties (Olson *et al.,* 1956). Acto-myosin preparations from failing heart muscle of man show reduced contractility (Kaks and Bing, 1958).

In thyrotoxicosis, with high-output cardiac failure, there is a greatly increased total oxygen consumption, increased coronary blood flow and augmented oxygen usage (Rowe *et al.,* 1956). But the threshold for glucose extraction by the muscle cells is raised, the extraction efficiency for pyruvate and lactate is reduced. Hence dependence upon fatty acid catabolism for energy becomes more and more imperative in hyperthyroidism. Myosin abnormality has not been demonstrated.

Myocardial anoxia is extremely severe in *ventricular fibrillation.* Cardiac output and coronary flow are greatly reduced and metabolism of the heart muscle is much altered (Bing). Glucose, pyruvate, lactate and potassium loss from the muscle is considerable and quite likely certain co-enzymes such as ATP are maintained in their phosphorylated form only as long as active oxidation is assured. When it fails the co-enzymes break down and the process becomes irreversible.

Response of the heart muscle to ischaemia obviously is not uniform. The classical response with lactic acid production in the muscle by way of the usual glycolytic mechanism is obtained only in its severest form, as with ventricular fibrillation. When ischaemia is moderate the metabolic defect may be confined to the level of the co-enzyme co-carboxylase.

REFERENCES

Bailey, K. (1956) : *Brit. med. Bull., 12:*183.

Bendall, J. R. (1951) : *J. Physiol. Lond., 114:*71.

Bing, R. J. (1956) : *The Harvey Lectures.* 1954–5. Ser. 50, 27. New York.

Greiner, T. (1952) : *J. Pharm. exper. Therap., 105:*178.

Hackel, D. B., Goodala, W. T. and Kleinerman, J. (1954) : *Circulat. Res., 2:*169.

Hodgkin, A. L. (1951) : *Biol. Rev., 26:*339.

Huckabee, W. E. (1958) : *J. Clin. Invest., 37:*244.

Huxley, H. E. (1956) : *Brit. med. Bull., 12:*171.

Kako, K. and Bing, R. J. (1958) : *J. clin. Invest., 37:*465.

Lipmann, F. (1941) : *Advances in Enzymol., 1:*99.

Olson, R. E., Ellenbogen, E., Stern, H. and Liang, M. M. L. (1956) : *J. clin. Invest., 35:*727.

Olson, R. E. and Patnek, D. A. (1959) : *Ann. N. Y. Acad. Sci., 72:*466.

Olson, R. E. and Schwartz, W. B. (1951) : *Medicine, 30:*21.

Rowe, G. G., Houston, H. H., Weingstein, A. B., Tuchman, H., Brown, J. F. and Crumpton, C. W. (1956) : *J. clin. Invest., 35:*272.

Szent-Gyorgyi, A. (1953) : *Chemical Physiology of Contraction in Body and Heart Muscle.* New York.

Szent-Gyorgyi, A. (1956) : *Fortschrit. Kardiol., 1:*6

Yokayama, H. A., Jennings, R. B., Clabaugh, G. F. and Wartman, W. B. (1955) : *Arch. Path., 59:*347.

Chapter 9

AUTOLYSIS

When cells die they sooner or later lose their respiratory and general metabolic functions, cease to move and reproduce, become more acid in reaction and alter their permeability characteristics in relation to macromolecules and ions. Hence their protoplasm swells because of increased water intake and their mitochondria and later their nuclei become water-logged. Granular bodies appear, cytoplasm and nucleus coagulate, and later lyse and disintegrate into debris (Cameron, 1952). Most, if not all, of these disturbances have long been held to be the outcome of unrestricted enzymic action, even when bacterial contamination is prevented. The term autolysis refers to that phase of tissue and cell death in which cellular enzymes play the chief role in breakdown, but there is nothing unique in the set of changes, for they are encountered in all varieties of necrosis.

MORPHOLOGICAL CHANGES DURING AUTOLYSIS

If a piece of liver, say from a mouse, is allowed to autolyse in a sterile container under conditions closely approximating those that hold when the animal dies, little happens until about six hours have elapsed (Berenborn *et al.*, 1955). Some nuclei then show pyknosis with disintegration of their membrane but the cytoplasm is still apparently unaffected. Soon afterwards mitochondria begin to swell and vacuolation of cytoplasm commences (Holle *et al.*, 1955). At twenty-four hours karyolysis is pronounced in areas deeply placed in the tissue while hyperchromatic nuclei are numerous everywhere. Most cells show reduced amounts of chromatin, nuclear membranes are indistinct and scattered cells are breaking up into granular clumps. By forty-eight to seventy-two hours most of the cells are completely necrotic.

Similar changes take place after natural death but they are soon complicated by bacterial growth and putrefaction.

CHEMICAL CHANGES IN AUTOLYSING CELLS

Autolysing liver shows a steady decrease in its protein nitrogen, RNA and DNA up to forty-eight hours of the onset of autolysis, after which they remain relatively constant. RNA and DNA fall to about six to seven and twelve per cent respectively of their original content. Free amino acids, acid soluble N, acid-soluble P increase up to forty-eight hours, then decline. Lipid content is least changed.

There is a general loss of cellular enzymes. Thus succinoxidase and cytochrome oxidase activity disappear in twenty-four hours and acid and alkaline phosphatase, esterase and peptidase activity continues to decline over seventy-two hours. The decreased rate of removal of protein after forty-eight hours reflects the declining activity of proteolytic enzymes and this is indicated to some extent by the fairly rapid disappearance of L-leucylglycine peptidase (Berenborn *et al.,* 1955; Wahi *et al.,* 1955; Gössner, 1955).

Release of enzymes from the cell lysosomes has been recently recorded by de Duve and his co-workers (1959). These minute organelles are present in cell cytoplasm and are of similar size to mitochondria but are distinct from other intracellular particles. In freshly isolated preparations they behave as fragile but intact sacs. When they are injured they release a collection of soluble hydrolytic enzymes whose activity is maximal at acid reaction. Disruption of their lipoprotein membrane is no doubt responsible for the enzymic leakage. In the ischaemic rat liver, for instance, there is progressive release of the lysosomal hydrolases, acid phosphatase, cathepsin, β glucuronidase, acid ribonuclease and acid desoxyribonuclease. This release reaches a plateau corresponding to the rupture of about 80 per cent of the lysosomes in three to four hours. Cytochrome oxidase activity is reduced to one tenth in six to eight hours, glucose-6-phosphatase about the same in ten to twelve hours, cathepsin and the two acid nucleases reach fifty to sixty per cent activity after about 8 hours, β glucuronidase is unaffected for six hours, then increases by about 40 per cent during the next twelve hours and remains elevated while acid phosphatase remains unchanged during the first day and then slowly decreases to about half its value during the second day. Ischaemic release of hydrolases may be the outcome of an anoxic change in the intracellular environment of the lysosomes which is quite likely reflected in a lowering of pH. Possibly there is a primary attack on the lysosomal membrane with extension of autolysis from these centres, as the released enzymes diffuse away. Lowering of pH, which is known to accelerate the breakdown of lysosomes *in vitro*, may involve the particles' own cathepsin acting on the membrane from the inside. Mitochondrial cytochrome oxidase and microsomal glucose-6-phosphate also are inactivated during autolysis. De Duve records release of lysosomal hydrolases in the liver of rats after starvation, poisoning by carbon tetrachloride, dietary deficiency, ligation of the common bile duct, especially when the animals are comatose. All such results, he believes, confirm the hypothesis that the opening of lysosomes, and the consequent release of their internal enzymes, plays an important part in the intiation of autolysis. Enzymic alterations may precede by several hours the manifestation of well-defined microscopical lesions.

Our information about autolysis in other organs is extremely limited, but where available it agrees fairly well with that obtained from liver investigations. Thus, the autolysing or ischaemic kidney loses succinic dehydrogenase, phosphatases, esterases and lipases, the brain, phosphatases. But the subject badly needs re-investigation with modern techniques.

In our own laboratory factors influencing the survival of liver cells during autolysis have been studied by the artificial perfusion technique applied to the rat (Gallagher *et al.*, 1956; Dawkins, Judah and Rees, 1959) . Previous investigations had convinced our colleagues that early autolysis is accompanied by a great increase in wet weight and accumulation of calcium ions in the cells. At this time cloudy swelling and vacuolation of the cytoplasm are the usual microscopical findings. Dawkins *et al.*, attributed these changes to a failure of the liver cells to maintain osmotic balance because of an inadequate quota of ATP. Homogenates prepared from such autolysing livers certainly fail to couple oxidation with phosphorylation. This topic is dealt with elsewhere (See "Enzymes," Chapter 5) .

REFERENCES

Berenborn, M., Chang, P. I., Betz, H. E. and Stowell, R. E. (1955): *Cancer Res., 15*:1.

Cameron, G. R. (1952): *Pathology of the Cell,* pp. 295–303. Edinburgh & London.

Dawkins, M. J. R., Judah, J. D. and Rees, K. R. (1959): *J. Path. Bact., 77*:257.

de Duve, C. and Beaufay, H. (1959): *Biochem. J., 73*:610.

Gallagher, C. H., Judah, J. D. and Rees, K. R. (1956): *J. Path. Bact., 72*:247.

Gössner, W. (1955): *Arch. path. Anat., 327*:304.

Holle, G., Burckhardt, R., Arndt, S. and Bloedorn, M. (1955): *Arch. path. Anat., 327*:150.

Wahi, P. N., Tandon, H. D. and Bharadwaj, T. P. (1955): *Acta path. microbiol. Scand. 37*:305.

Chapter 10

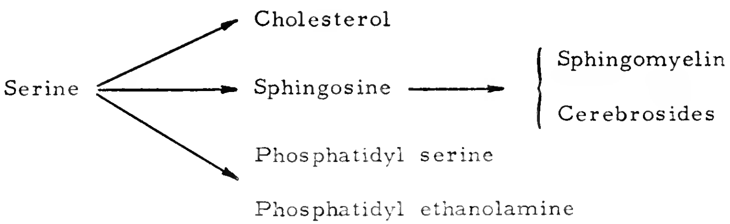
DEMYELINATION

WE OFFER NO APOLOGY for including a discussion of the myelin sheath of nerve fibres in our programme, for it is very frequently the site of serious damage that cannot be separated from the nerve and neuroglial cells whose existence is so closely linked with the preservation of that sheath. Demyelinating diseases still offer the supreme challenge to the neurologist; they afford the investigator a unique opportunity for exact chemical study of a unique cellular function.

Electron microscopic and x-ray diffraction studies show the myelin sheath to be a highly organised structure in which an orientated double layer of lipid alternates with a protein layer (Schmitt, 1939; Schmitt and Palmer, 1941) . Cholesterol is an integral part of this lipoprotein complex (Fernandez-Moran and Finean, 1957) . The sheath is made up of a number of lamellae each about 140 Ångstrom units wide and varying from one or two in "unmyelinated" nerve to thirty-six or more layers in the myelinated nerve. Modern investigations have established an intimate relationship between the myelin and the Schwann cells that are grouped about the peripheral axons. It appears that the lamellae of myelin are wound around the axon layer by layer, like a bandage, the ends remaining attached on the inside to the axolemma and on the outside to the Schwann cell membrane (Lumsden, 1957) . There is little doubt that the Schwann cell is responsible for this winding process but how it does it is still debated. Confirmation has come from the tissue culture studies of Peterson and Murray (1955) who have adroitly followed myelination *in vitro* though they could not decide whether lipid comes from the axon proper or the Schwann membrane. A similar mechanism quite likely holds between the oligodendrocytes and the axons of the central nervous system.

CHEMISTRY OF MYELINATION

The myelin sheath is composed largely of lipids radially orientated in the more or less concentrically arranged lipoprotein laminae. The lipid components include cholesterol, sphingomyelin, cerebroside and probably phosphatidyl serine. Sphingomyelin is composed of fatty acid—probably C_{24} lignoceric acid or stearic or neuronic acid—sphingosine and phosphoryl choline. Cerebroside is a component of fatty acid, sphingosine and hexose. The sphingosine-containing lipids are among the most characteristic constituents of the myelin sheath. Serine is an effective precursor of sphingosine. After parenteral injection of [3-^{14}C] serine into very young rabbits radio-active cholesterol, sphingomyelin, lecithin, cerebroside and cephalins can be recovered from their brain, spinal cord and sciatic nerves. There is little change in the radioactivity of these compounds over the next 192 days so that it seems that once deposited in the myelin sheath at the time of its formation they undergo little subsequent turnover. (Davison *et al.*, 1959).



So, too, radio-active-C labelled cholesterol when injected into the yolk sac of newly hatched chicks disappears much slower from the brain than from the liver and is still present in the central nervous system after 320 days (Davison *et al.*, 1958, 1959). A slow turnover has also been demonstrated in the grey matter of the brain of young rabbits, but not in the white matter.

WALLERIAN DEGENERATION OF THE PERIPHERAL NERVES

When a peripheral nerve is divided or crushed it undergoes Wallerian degeneration. The axon disintegrates and disappears while the myelin sheath at first fragments and ultimately is destroyed. Schwann cells proliferate and the nerve sheaths thicken to become Schwann tubes along which grow the new axon tips from the intact central stump.

Myelination spreads down the nerve in a peripheral direction and eventually the myelin sheaths of the regenerated nerve fibres are closely similar to those of a normal nerve.

After section the water content of the nerve increases, reaching a maximum, in the cat, in thirty-two days and returning to normal by 144 days. The total lipid content of the nerve decreases during the first sixteen days, remains constant up to the forty-eighth day and then increases gradually. Even after 144 days the concentration of total lipid is less than that of corresponding normal nerves. Neutral fat decreases rapidly but is normal once again by forty-eight days. The myelin lipids—cerebroside, free cholesterol and sphingomyelin—decrease during the first thirty-two days, remain constant until the ninety-sixth day after which they increase gradually. By 144 days myelin-lipid concentration in the cat's sciatic nerve is 44 per cent of the normal value. Both total and free cholesterol decrease during the first thirty-two days, free cholesterol more rapidly than total, for cholesterol ester, absent in normal control nerves, appears during this period. Free cholesterol behaviour parallels that of the myelin lipid but cholesterol disappears by 144 days. Total phospholipin decreases during the first thirty-two days, then remains fairly constant until after ninety-six days when it increases gradually. Sphingomyelin follows a similar course, but cephalin decreases more rapidly and lecithin more slowly. The concentration of the latter is similar at 144 days to that at sixty-four days (Burt *et al.,* 1950). These changes are very like those that occur during autolysis. They suggest that a phospholipid-splitting mechanism is concerned in both cases.

DEMYELINATION

Demyelinating diseases are common disorders of the central nervous system in which the myelin sheath of the white matter is lost. They have many causes that range from vascular occlusion leading to cerebral softening, neoplastic compression, Wallerian degeneration due to loss or damage of the neurones, to ascending or descending tract degeneration. The commonest chronic type of demyelinating disease is disseminated or multiple sclerosis. No general agreement about the true nature of demyelinating processes exists and the suggested causes include a variety of microorganisms, viruses, deficiencies and intoxications and injuries (McAlpine, Compston and Lumsden, 1955). Demyelination has been produced in experimental animals by chemical agents such as carbon monoxide, potassium and sodium azide, by various toxins such as ergot, bee venom, tetanus toxin, organic phosphorus compounds and by homologous brain suspensions emulsified with the heat-killed acid-fast bacilli (Freund adjuvants). Demyelinating diseases of lambs (Swayback) are in some way related to copper deficiencies in the pastures on which the pregnant ewes have been grazed before lambing. A similar association has not been recorded in man.

Changes in lipid composition of the central white matter may be illustrated by the following table compiled by Cumings from cases of multiple sclerosis.

CEREBRAL LIPIDS IN MULTIPLE SCLEROSIS

Lipids	*Normal white matter*	*Demyelinated matter*
Total phospholipid	5.8	1.8
Sphingomyelin	2.4	0.5
Total cholesterol	4.1	2.8
Esterified cholesterol	0.3	1.7
Ganglioside	. . .	1.0
Water	69 per cent	80.4 per cent

Results in grams per 100 g. fresh tissue except for ganglioside which is in grams per 100 g. dry tissue.

Demyelinated white matter shows a marked decrease in total phospholipid and total cholesterol with a very great increase in esterified cholesterol.

So far the cause and pathogenesis of multiple sclerosis and other demyelinating diseases of man have eluded the investigator. A little progress has come from animal studies which may in time prove to be of some importance for the human problem.

Both Swayback and the closely related condition enzootic ataxia which occur frequently in Australian lambs are associated with a deficiency in copper. In Australia this deficiency is due to the lowered level of copper in the pasture, but in Great Britain Swayback pastures contain adequate amounts of copper and it is thought that the disease is caused by a failure in the intermediary metabolism of copper in the ewe or by the copper in the pasture being in an unavailable form (Hunter *et al.,* 1945). Our colleagues Gallagher, Judah and Rees (1956) have produced copper deficiency in young rats and hens and shown that the copper content of the liver, kidney and brain is then reduced although demyelination cannot be demonstrated. Growth is retarded, the young rats become anaemic and there is reduced pigment formation in the skin. The latter quite likely is the result of deficiency of tyrosinase which requires copper as its prosthetic group. The anaemia is associated with decreased activity of cytochrome oxidase which also requires copper. In addition, our colleagues have discovered an impairment in the synthesis of phospholipid in their copper-deficient animals possibly due once again to deficiency of an enzyme that requires copper for its activity. Since myelin is composed largely of phospholipids and synthesis goes on very actively during the first few days of life we may have, in this discovery, a suggestive pointer for further metabolic research into demyelinisation. Already it has been shown that the copper content of the brain and liver from Swayback lambs is lower than normal and that the copper-dependent enzyme cytochrome oxidase in the brain of these lambs is also reduced (Howell and Davison, 1959).

Further studies are needed along these lines with special attention given to the Schwann cells and the oligodendrocytes whose function it is to lay down the myelin sheath. It is to be hoped that close cooperation between the biochemist, tissue culturist and neurohistologist will yield fundamental information that may solve the problems of these elusive diseases.

REFERENCES

Burt, N. S., McNabb, A. R. and Rossiter, R. J. (1950): *Biochem. J., 47:* 318.

Cumings, J. N. (1959) : *J. clin. Path., 12*:489.

Davison, A. N., Dobbing, J., Morgan, R. S. and Payling Wright, G. (1958) : *J. Neurochem., 3*:89; (1959) : *Lancet, i*:658.

Davison, A. N., Morgan, R. S., Wajda, M. and Payling Wright, G. (1959) : *J. Neurochem., 4*:353.

Fernandez-Moran, H. and Finean, J. B. (1957) : *J. biophys. biochem. Cytol., 3*:725.

Gallagher, C. H., Judah, J. D. and Rees, K. R. (1956) : *Proc. Roy. Soc. B., 145*:134, 195.

Howell, J. M. and Davison, A. N. (1959) : *Biochem. J., 72*:365.

Hunter, A. H., Eden, A. and Green, H. H. (1945) : *J. comp. Path., 55*:29.

Lumsden, C. E. (1957) : *Modern Trends in Neurology,* (2nd series). Chaps. 10 & 11. London.

McAlpine, D., Compston, N. D. and Lumsden, C. E. (1955) : *Multiple Sclerosis.* Edinburgh & London.

Peterson, E. R. and Murray, M. R. (1955) : *Amer. J. Anat., 96*:319.

Schmitt, F. O. (1939) : *Physiol. Rev., 19*:270.

PART III

CHEMISTRY OF THE REACTION TO INJURY

Chapter 11

INFLAMMATION AND RELATED PHENOMENA

INFLAMMATION

INFLAMMATION IS THE local response of small blood vessels to injury. It is characterised to the naked eye by redness, swelling, increased heat and pain in the affected part. Microscopically it is characterised by dilatation of arterioles, capillaries and venules, by increased permeability of venules and capillaries to plasma protein and by adhesion of leucocytes to the vascular endothelium and the emigration of leucocytes into the tissues. Inflammation is a cyclical reaction in which changes in vascular calibre and permeability precede leucocyte emigration from vessels. The predominantly polymorphonuclear exudate may be in turn replaced by lymphocytes, macrophages and sometimes plasma cells. The exudate may then disappear, leaving the tissue normal once again or undergo organisation in which there is replacement of the damaged area, by collagen laid down by fibroblasts.

The similarity of the inflammatory cycle in many different species in response to many diverse types of injury, including transient physical damage has led to the now generally accepted view that the vascular events of inflammation are due at least in part to the release or activation of local hormones or mediators (see Spector, 1958). This hypothesis gained support from the discovery of naturally-occurring substances with effects on small blood vessels similar to those seen in inflammation. In recent years the theory has been strengthened further by the demonstration of active forms of such compounds at the site of injury at the time when they should be exerting their effect. Additional evidence has been provided by the experimental suppression of inflammatory changes with the aid of specific inhibitors of compounds suspected to be chemical mediators of these changes.

HISTAMINE

Histamine (Wolstenholme and O'Connor, 1956) is a di-amine derived from the amino acid histidine. It is very widely distributed in the tissues of all mammals. It is formed by the enzyme histidine decarboxylase and destroyed by di-amine oxidase (histaminase). Histamine dilates capillaries and increases their permeability to protein and in some species dilates arterioles; it is thus an excellent candidate for the role of mediator in inflammation. Lewis drew attention to the similarity of the action of histamine and the vascular events of early inflammation and postulated the release by injury of an histamine-like substance.

Later studies showed that histamine was in fact liberated from tissues damaged by a variety of means, particularly antigen-antibody reactions but including trauma, burns and infection. Histamine was demonstrated in the circulation of the injured animals and in the effluent from perfused tissues. It was also shown that a large variety of organic bases could cause massive release of histamine from tissues in the absence of apparent cellular damage (Spector, 1958). There is also evidence that injury may cause increased activity of histidine decarboxylase and thus lead to increased synthesis of histamine (Schayer, 1960). The discovery of a group of drugs that antagonised more or less specifically the effects of histamine led to further advances. Treatment with these compounds greatly reduced the inflammation caused by antigen-antibody reactions in certain situations, e.g. allergic rhinitis and urticaria. However, the majority of inflammatory lesions both natural and experimental were not affected by the anti-histamine drugs. As a result it seemed that histamine had little or no part in the inflammatory reaction in general.

Some experiments conducted at University College Hospital helped to resolve this discrepancy. When turpentine is injected into the rat's pleural cavity, there is very rapid formation of an inflammatory exudate. In the first thirty minutes, samples of this exudate contain significant quantities of histamine but after this time no histamine can be detected. This result suggests that histamine plays its part early in the inflammatory reaction and that other mechanisms may then come into operation.

This hypothesis was confirmed with the aid of anti-inflammatory drugs (Spector, 1958). When animals in which pleurisy has been induced with turpentine are pre-treated with small doses of an anti-histamine drug, the appearance of the exudate is delayed for one or two hours. After this delay the exudate develops normally, even if repeated doses of the anti-histaminic are administered. The effect of anti-histamines could also be achieved by prior depletion of bodily histamine. A precisely similar result is obtained in numerous species when inflammation is induced by a standard thermal injury (Spector and Willoughby, 1959; Wilhelm, 1959). Here the passage of plasma from the vessels into the damaged tissues is again delayed by prior dosage with a specific anti-histamine drug. Again, too, repeated doses fail to prevent the subsequent appearance of the inflammatory cycle.

These results show that the role of histamine in inflammation is to initiate the vascular changes, especially increased capillary permeability, and that the subsequent sustenance of these changes is due to other mechanisms independent of histamine release. The rapidity with which the effects of histamine occur after injury may be explained by assuming that the amine is released from mast cells, which adjoin blood vessels in large numbers and which are rich in histamine. These cells are known to be disrupted and liberate their granules in response to injury. The precise mechanism of histamine release by injury is unknown. Uvnäs (1958) has suggested that the membrane of mast cells contains an enzyme capable of lysing the membrane and thus releasing the cellular content of histamine and that injury activates this enzyme. Other workers have suggested that injury leads to the formation of surface-active compounds, e.g. peptides which have a similar effect on the mast cell membrane. Apart from these ideas, the release of histamine has been postulated to follow rupture of a peptide or polar bond linking histamine to a protein, or an ion-exchange reaction releasing histamine from loose combination with an acidic body compound. Mast cells contain a trypsin-like enzyme and there is some evidence that release of histamine from these cells depends upon activation of this protease (Glennor and Cohen, 1960).

5-HYDROXYTRYPTAMINE (5-H.T., SEROTONIN)

This substance is a monoamine derived from the amino acid tryptophane. Like histamine, it is widely distributed in animal tissues and is thought to play a role as a neurohormone in the brain. Like other monoamines it was thought to be a vasoconstrictor, but Rowley and Benditt (1956) showed that in very low concentrations in the rat it increased capillary permeability, i.e., had a histamine-like action. In most other species, however, 5-H.T. does not increase capillary permeability, so that any role it plays as a mediator of inflammatory changes must presumably be confined to the rat and perhaps the mouse. Compounds exist more or less specifically antagonistic to 5-H.T. Dosage with such substances considerably reduces the increased capillary permeability caused in the rat by injection of certain large molecular substances, e.g. egg white or dextran, suggesting that release of 5-H.T. is in part the cause of this particular type of vascular reaction. It is known also that in the rat, histamine liberators such as compound 48/80 release 5-H.T. too. However, administration of 5-H.T. inhibitors to rats subjected to injury caused by turpentine, heat and x-rays has failed to influence the course of inflammation. Thus no evidence yet exists that 5-H.T. is important in true inflammation even in the rat. In spite of these findings, there is ample evidence for the release of 5-H.T. by a variety of injuries in a number of species, an observation whose significance is yet to be revealed (Spector, 1958). The substance might be liberated as a result of damage to platelets (which occurs in the early stages of tissue injury), or lysis of mast cells, and perhaps play a role in the causation of pain if not capillary permeability (see below).

PEPTIDES

Peptides consist of varying numbers of amino acids joined by peptide linkages and forming molecules too small to be considered as proteins. Their possible role in inflammation has been suspected for a hundred years, but the study of the effect of peptides on capillaries really dates from some observations of Menkin (1956). Menkin showed that albumin partially digested with trypsin increased capillary permeability and that the property was likely to reside in the peptide fraction. Later work demonstrated that large numbers of different peptides from various sources were able to increase capillary permeability and that the most likely requisite for this property was a molecule containing eight to twelve amino acids. In addition, pharmacologists began to describe a variety of individual peptides which they found in urine, blood and tissue extracts and that increased capillary permeability (see Spector, 1958). These observations culminated recently in the purification of one of the most important members of the group, bradykin, a peptide derived by proteolysis from plasma globulin. In conformity with the earlier analyses, this substance was found to contain eight amino acids in its molecule. All peptides that affect capillary permeability have certain common properties. They stimulate plain muscle, e.g. of uterus and intestine, behave as chemical bases on electrophoresis, tend to be hypotensive in dogs and cats, to cause pain on application to a blister base and to be inactivated by chymotrypsin. Those derived from blood plasma usually originate in the α^2 globulin fraction. It is now customary to refer to peptides of this type as kinins.

It is thus obvious that peptides are capable of the role of chemical mediator of altered capillary permeability after injury. Unfortunately, it has proved difficult to demonstrate their presence at the time and place needed to establish their causative role. One possible exception is provided by stimulation of the nerve supplying salivary gland tissue. Such stimulation leads to inflammatory-like changes in the vessels of the gland and also to the appearance of kinins in the effluent from the gland (Hilton and Lewis, 1955). It is very possible that much of the difficulty hitherto experienced is due to rapid destruction of the peptides by peptidases in blood and tissues. It is known that kinins activated in plasma, e.g. by contact with glass, are destroyed in a few minutes by such enzymes, and it seems likely that peptides activated by tissue injury would enjoy an equally transient existence. Moreover, there is no reason to suppose that rapid destruction in plasma would prevent their effect on capillaries in inflammation. Destruction by peptidases may in fact be a homeostatic mechanism for inactivating these potent substances once they escape from the site of the inflammatory reaction. Since there are no specific inhibitors of the formation and activity of kinins it is as yet impossible to test their role in inflammation by the administration of such compounds.

PROTEINS

There exists in the globulin fraction of the plasma proteins of all mammalian species yet tested, a substance capable of increasing capillary permeability in very low concentration (Miles and Wilhelm, 1955; Wilhelm *et al.*, 1958). This globulin is present normally as an inert precursor that can be activated by a variety of procedures including dilution with saline, contact with organic solvents, and incubation with minced tissues. It is therefore an obvious candidate for the role of mediator of the vascular changes of acute inflammation. The protein could act directly on vessels (possibly enzymically) or could be either protease or, less likely, substrate in the formation of peptides of the kinin type.

A substance with properties similar to those of the plasma globulin can be obtained by extracting a variety of minced tissues, e.g., lung, skin or spleen with isotonic saline. The extracts contain in active form a powerful capillary permeability factor. It is not yet certain whether the active principle of these tissue extracts is identical with the substance obtained from plasma but they have up till now been assumed to be of similar nature. It is also known that certain mucoproteins, derived for example from glandular secretions of the guinea pig are highly active in increasing capillary permeability. In the case of all these proteins it is not yet known whether their action is direct or whether they exert their effect by giving rise to kinins. However, it is established that certain enzymes of the protease and esterase group, will cause increased capillary permeability on injection into the tissues of living animals quite possibly by a direct action on the vascular wall. Trypsin is one such enzyme, but as this substance is a histamine liberator, its action on capillaries may not be related directly to its proteolytic function. Another such enzyme is kallikrein which is present in various tissues and body fluids and which is believed to exert its effects on capillaries and smooth muscle by catalysing the formation of kinins from globulin substrates (see also Spector, 1958).

Study of inflammatory pleural exudates produced with the aid of turpentine has shown that exudates obtained at the height of increased capillary permeability contain activated permeability-increasing globulins but that as the inflammation subsides, the globulin in the exudate is present entirely in the inactive form. This change is due in part to the relative preponderance of a specific inhibitor of the globulin normally present in plasma (Spector, 1958).

There is evidence that proteases may be activated by injury although some of the evidence is conflicting (Spector, 1958). Anaphylaxis certainly leads to protease activation in the blood of dogs, and a skin protease may be demonstrated after thermal injury. Tissues contain at least two enzymes that split globulins to yield kinins, one rapid in its action and one slow (Lewis, 1959). However, it seems that kallikrein rather than plasmin is likely to be the enzyme responsible for the formation of kinins after injury and it is possible that the activity of permeability-increasing globulins is in fact due to kallikrein (Bhoola, Calle and Schachter, 1960).

It has already been stated that permeability-increasing globulins are converted to an active form by incubation *in vitro* with minced tissues or isolated mitochondria. This activation is prevented by the presence of a number of compounds including heparin, soya bean trypsin inhibitor and salicylate, quinine and di-isopropyl fluorophosphate (DFP). Administration of some of these compounds, i.e. salicylate, quinine and DFP prevents the development of increased capillary permeability after thermal or chemical injury. These results might be taken as confirmation of the part played by activated globulins in maintaining the inflammatory reaction following the release of histamine. This is particularly the case when considering the action of salicylate in turpentine pleurisy since the drug suppresses the early stages of exudate formation (thought to be due to histamine) much less than the later stages possibly due to activated globulins or peptides (Spector, 1958). This interpretation may still be correct, but unfortunately from the point of view of simplicity, compounds that inhibit globulin activation also cause a general suppression of capillary permeability increased by a variety of compounds (Spector and Willoughby, 1960a), an action discussed in the pages that follow.

THE INACTIVATION OF VASOCONSTRICTOR AMINES AS PART OF THE INFLAMMATORY REACTION

The vascular events of acute inflammation may be regarded as a temporary disturbance of the balance of forces normally governing the behaviour of vessels, the vasoconstrictor forces being for the time in abeyance. So far only influences actively dilating vessels and increasing their permeability have been considered. Recently, however, evidence has come to light supporting a new concept, namely that the vascular changes of acute inflammation are partly due to the destruction of an amine that would otherwise constrict and reduce the permeability of capillaries and oppose the action of compounds such as histamine and kinins.

The most important endogenous compounds with these "anti-permeability" actions on blood vessels are, the catechol monoamines adrenaline and nor-adrenaline, (epinephrine and norepinephrine). Amongst other situations, they are present in platelets, leucocytes and vessel walls and at least two enzymes destroy them in the body, monoamine oxidase and catechol-o-methyl transferase. It has been found recently at University College Hospital that the administration of specific inhibitors of monoamine oxidase greatly reduces the increased capillary permeability consequent on thermal or chemical injury. This result is explicable on the basis that inflammatory phenomena are in fact partly due to inactivation of vasoconstrictor amines by monoamine oxidase (Spector and Willoughby, 1960b). Administration of competitive inhibitors of catechol-o-methyl transferase failed to modify the inflammatory reaction.

The inhibitory action of monoamine oxidase inhibitors on the inflammatory reaction is abolished by administration of dibenamine and similar compounds that inactivate adrenaline and related amines. Moreover, the action of monoamine oxidase inhibitors is potentiated by bretylium tosylate, a substance that augments the effects of circulating adrenaline. In addition, the effects of monoamine oxidase inhibitors on inflammation can be reproduced by injection of adrenaline although not of nor-adrenaline or 5-H.T. Finally, the effects of monoamine oxidase inhibitors are not abolished by prior adrenalectomy (Spector and Willoughby, 1960b) or hypophysectomy (Setnikar *et al.*, 1959). These results indicate that following injury, adrenaline-like substance and oxidase may be brought into contact. As a result, the vasoconstrictor amine may be destroyed, and inflammation allowed to proceed. It seems possible that in the wall of the normal small blood vessel, adrenaline-like and histamine-like compounds compete for receptor sites, the interplay of their actions making for normal vascular reactions. In inflammation, not only are the vasodilator forces greatly augmented (as described above) but the vasoconstrictor forces may be inactivated. The enzymic inactivation of the adrenaline-like substance could be precipitated either by local activation of monoamine oxidase or release of the amine from a site inaccessible to the enzyme.

THE MECHANISM OF INCREASED CAPILLARY PERMEABILITY IN INFLAMMATION: A SUMMARY

Figure 10 attempts to illustrate the picture of vascular events in inflammation as outlined in the preceding pages. It is suggested that the initial event is a release of histamine from mast cells, platelets or other sources by a mechanism not yet fully understood but possibly involving lytic enzymes in the mast cells. This release may be prevented experimentally by previous "depletion" of body histamine with the aid of repeated injections of powerful histamine liberators such as compound 48/80. The effects of the histamine released by injury may be prevented by administration of small doses of anti-histamine drugs such as Anthisan (Neoantergan). Histamine appears to exert its effects within a minute or two of injury and continues to dilate capillaries and increase their permeability for at least one to two hours.

It is postulated that at the same time as histamine is released, an adrenaline-like substance is brought into contact with the enzyme monoamine oxidase which destroys it. This reaction may be prevented by administration of monoamine oxidase inhibitors. The effects of these inhibitors suggest that the hypothetical destruction following injury of an adrenaline-like substance leads to dilatation and increased permeability of small vessels that begins within a few minutes and may last for 24 hours or even longer.

Soon after these initial events there is activation of globulins and peptides that increase capillary permeability. The mechanism is unknown but may involve the action of enzymes of the esterase-

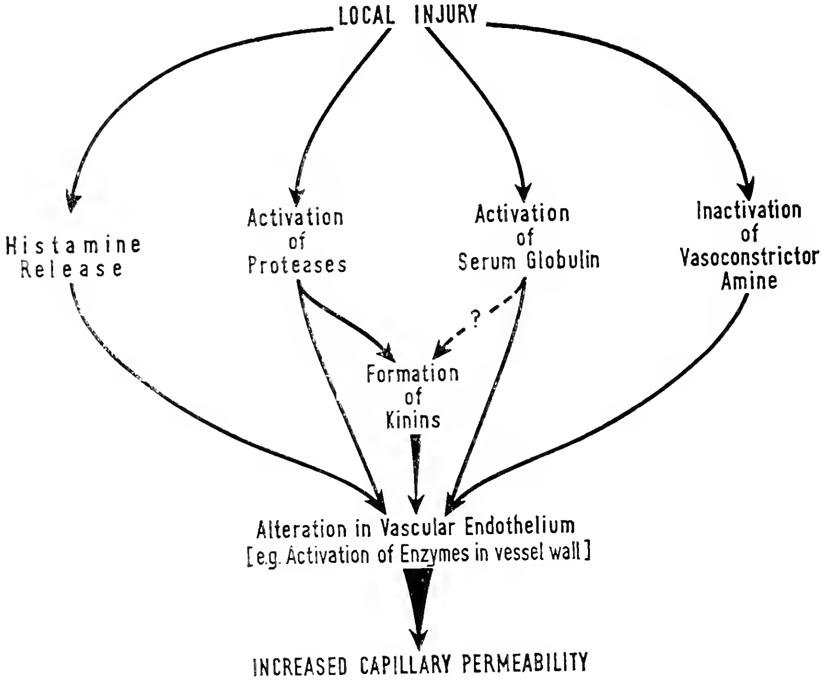

LOCAL INJURY
Histamine Release
Activation of Proteases
Activation of Serum Globulin
Inactivation of Vasoconstrictor Amine
?
Formation of Kinins
Alteration in Vascular Endothelium
[e.g. Activation of Enzymes in vessel wall]
INCREASED CAPILLARY PERMEABILITY

Fig. 10. Schematic Version of the Mechanism of Increased Capillary Permeability in Inflammation.

protease group. This activation may possibly be prevented by substances which antagonise these enzymes *in vitro,* e.g. salicylate, quinine or DFP. The effect of globulins and peptides on blood vessels does not become apparent until one-half to two hours after injury.

Little is known of the intimate mechanism whereby histamine, globulins and peptides increase capillary permeability. However, there is some evidence for a final common pathway since histamine renders capillaries partially refractory to the action of peptides and since all direct-acting capillary permeability factors have certain common properties, e.g. free amino groups, action on smooth muscle. The nature of this final common pathway may possibly be indicated by the fact that inhibitors of the esterase-protease group of enzymes, notably salicylate and quinine, suppress the increased capillary permeability induced by local injection of histamine, 5-H.T., globulins, peptides, and histamine releasers. Thus there is some evidence that all endogenous mediators of increased capillary permeability may exert their effect by activating an enzyme of the esterase-protease group in or near the vessel wall (Spector and Willoughby, 1960a). The substrate of this enzyme could be a protein or phospholipid in the capillary wall or the precursor of yet another mediator substance which then acts on the blood vessel. It is known that peptides are capable of activating an esterase in guinea pig serum (Becker *et al.*, 1959) and an esterase capable of splitting synthetic substrates, e.g., α naphthyl acetate has in fact been demonstrated in or around the walls of small blood vessels by histochemical means. Inhibitors of monoamine oxidase do not lessen the effect on capillaries of histamine, globulins and peptides so that it is clear that these permeability-increasing compounds do not act by facilitating the enzymic destruction of the adrenaline-like substance. On the other hand, in inflammation induced by thermal injury the inhibitory actions of monoamine oxidase inhibitors and of anti-esterases such as quinine are not additive. This may mean that destruction of adrenaline-like substance may affect capillaries by way of the same common pathway as is followed by histamine, globulins and peptides. It is of interest that high concentrations of some anti-histamine drugs e.g. phenergan (promethazine HCl) not only exert a general antagonism to increased capillary permeability but also cause a general inhibition of electrolyte movements in damaged cells and mitochondria. This may possibly mean that increased capillary permeability to protein is in some way secondary to, or at least associated with, electrolyte disturbance in the vascular endothelium and that capillary permeability factors such as histamine may act by altering the electrolyte and water balance of these cells. Such speculations are rendered more plausible by recent electron microscopy which has indicated that protein appears to leave histamine-treated vessels by transport through the endothelial cytoplasm rather than by passage through channels of molecular dimensions between the cells, as was widely supposed (Alksne, 1960).

Because of many uncertainties it is appropriate to stress the hypothetical and tentative nature of the scheme for the mechanism of the vascular changes of inflammation presented in Figure 10. It cannot be denied that anti-inflammatory compounds such as salicylate, quinine or monoamine oxidase inhibitors may act in other ways, e.g. by stimulating the release of endogenous anti-inflammatory substances. Adrenaline itself may stimulate the pituitary gland to release adrenocorticotropic hormone (ACTH). However, in the experimental systems on which the above scheme is based adrenaline has a much more powerful anti-capillary permeability effect than any known adrenal cortical or pituitary hormone. In addition, careful studies have failed to provide evidence that these drugs, e.g. salicylate, do in fact exert their effect by stimulation of the adrenal cortex (Smith, 1959).

LEUCOCYTIC EMIGRATION

One of the early changes of acute inflammation is the adhesion of polymorphonuclear leucocytes of the blood to the luminal surface of the lining endothelium of small blood vessels. This phenomenon is followed in due course by the migration of polymorphs through the vessel wall into the tissues. The adhesion of leucocytes is associated in some way with the development of "stickiness" by the vascular endothelium. "Stickiness" can be demonstrated by the adhesion of colloidal particles injected into the circulation. It has also been shown to be associated with the aquisition by the endothelium of phagocytic properties, so that colloidal particles are ingested into the endothelial cytoplasm (McCluskey and Benacerraf, 1959). The development of stickiness and the associated phenomena of leucocyte adhesion and endothelial phagocytosis is probably due to changes at the surface of the endothelial cell. The essential feature of these changes may be loss of normal electrical negativity. Alteration in electrical charge presumably would be associated with modification of the radicles, e.g. COOH groups presenting at the cell surface, and such chemical alterations could well be more important than loss of electrical negativity alone. In any event the effect of these developments could be to inactivate those forces which normally prevent adhesion of leucocytes (which also carry a negative surface charge) and endothelium. The mechanism of adhesion may consist of aquisition of a coating of negatively charged protein by both cell surfaces, the two layers of protein then uniting with the aid of calcium ions.

The adhesion of numbers of potentially highly mobile polymorph leucocytes to endothelium together with further changes in the vessel wall allowing white cells to pass between the endothelium could account for much of the leucocyte emigration seen in acute inflammation. This argument is the basis of the first view of the mechanism of such migration. This theory suggests that the action of capillary permeability factors such as histamine, peptides and globulins is adequate to account not only for the passage of plasma into the tissues but also of leucocytes. There are three arguments in favour of this hypothesis. First, capillary permeability factors cause "stickiness' of vascular endothelium; second, injection of these substances in high concentration leads to leucocyte emigration from vessels; third, these permeability-increasing compounds do not appear to be chemotactic to leucocytes *in vitro* (Harris, 1954).

There are, however, powerful reasons for rejecting this theory as the sole cause of leucocyte emigration. Thus varieties of inflammation associated with comparable degrees of plasma exudation vary greatly in the extent of leucocytic infiltration of the tissues. In particular, certain bacterial infections, e.g. due to staphylococci lead to very intense accumulation of white cells outside the vessels.

Again, it is possible to obtain sterile tissue extracts, e.g. from the uterus of oestrogen-treated mice or from five hour old burns in rats, with a peculiarly powerful ability to induce leucocyte emigration, although the effect of such extracts on capillary permeability to protein is no greater than that of other tissue extracts with little action on leucocytes (Spector and Storey, 1958; Hurley and Spector, 1961).

Finally, in non-bacterial inflammation, the time course of increased capillary permeability to protein on the one hand and leucocyte emigration on the other, may be quite different. In thermal and chemical injury in rats the migration of polymorphs from vessels does not reach its peak until the passage of plasma into the tissues is declining.

In the case of the disproportionate tissue leucocytosis of certain bacterial infections, the leucocyte emigration may be attributed to chemotaxis. This implies that the living or dead organisms exert a positive directional "pull" on the white cells. The major evidence in support of this view is the powerful chemotactic effect of pus-producing organisms on leucocytes in carefully controlled systems *in vitro* (Harris, 1954). It may well be, however, that chemotactic influences in the tissues are unable to draw leucocytes out of vessels unless the vessel wall itself suffers some alteration such as occurs in increased capillary permeability. Thus capillary permeability factors and chemotactic stimuli might work together. It is the polysaccharide fraction of bacteria which is most likely to be the active principle of their chemotactic action on leucocytes. Polysaccharides which are chemotactic to white cells *in vitro* have been isolated from many strains of microorganisms and also from a variety of animal and plant tissues (Meier and Schär, 1957).

The discrepancies between increased capillary permeability and leucocyte emigration in non-bacterial inflammation cannot be resolved so easily. The extent of the discrepancy is revealed by some recent experiments by Hurley and Spector (1961) at University College Hospital. Small amounts of histamine injected intradermally into rats under conditions of rigid sterility are followed almost immediately by increased capillary permeability to protein which lasts for about 20 minutes. Sections of skin examined at this time and up to two hours later show no significant leucocyte emigration. However, similar sections examined five hours after injection of histamine show striking migration of polymorphs from small blood vessels in the injected area. Even more remarkable is the observation that a similar delayed leucocyte emigration occurs after injection of sterile isotonic saline which produces no demonstrable increase in capillary permeability to protein whatsoever.

It is clear that an explanation of this long latent period between disturbance of the environment of small blood vessels and leucocyte emigration must be sought in the affected tissues. Furthermore, the sequence of injury leading to increased capillary permeability, e.g. activation of plasma enzymes, breakdown of platelets, disruption of mast cells seem too rapid to be responsible for the delayed onset of leucocyte migration.

The answer to this problem may lie in the observation that soon after injection of histamine or saline, leucocytes may be seen adhering to the inner surface of small vessels. It seems possible that these white cells might slowly liberate a substance with the specific property of inducing emigration of further leucocytes on a massive scale. Such a process has been invoked to explain the liberation of pyrogenic substances from leucocytes following injection of bacterial endotoxin. In fact, extracts of leucocytes from blood and sterile exudates were found to contain a principle capable of inducing large-scale emigration of leucocytes within thirty minutes of their intradermal injection, a property not possessed by similar extracts of other types of body cells. Preliminary experiments indicate that a substance akin to that obtained from polymorph leucocytes may be activated in serum by incubation with minced tissues.

A substance with similar properties is present in the fluid portion of sterile exudates produced by intraperitoneal infusion of saline. In this instance the active principle might be derived from both disrupted leucocytes and activated plasma. Another source of the leucocyte emigration factor is rat skin when extracted several hours after thermal injury when exudation of polymorphs due to the burn is at its height. Here again, both sources may contribute to the activity of the extract. Fractionation of these skin extracts reveals much of the activity to reside in the fraction associated chiefly with albumin and α_1 globulin. The active principle is probably neither of these proteins but is more likely to be a highly soluble protein with a considerable carbohydrate component in its molecule. The substance is non-dialysable, relatively heat-labile, insoluble in 5 per cent trichloracetic acid and inactivated by trypsin. In its origin in leucocytes, its presence in the fluid phase of leucocyte-rich exudates and in skin several hours after injury, and its response to heating, the leucocyte emigration factor resembles the endogenous fever-producing substance demonstrated by American workers.

There are two possible modes of action for the leucocytic factor that induces polymorph migration. It might exert a chemotactic influence, the white cells being attracted through a vessel wall whose properties have been altered by other influences, e.g. histamine, globulins or peptides. Alternatively, the leucocyte migration factor might itself induce a change in vessel walls rendering them specifically permeable to white cells and this hypothesis seems the more likely. Accumulation of polymorphs around a bacterial colony or foreign body in the tissues is no proof of chemotaxis by the colony or foreign body, since the white cells may have arrived at their destination by random migration and then had their progress or their life terminated by toxic influences. The relative dissociation of vascular permeability to protein on the one hand and to leucocytes on the other suggested by the results presented above may seem remarkable. It may be explained, however, by assuming that much of the protein escapes into the tissues by passing through the cytoplasm of the endothelial cell (Alksne, 1959), whereas the leucocytes may leave the vessel by making their way between the endothelium. Thus two distinct but normally coexistent mechanisms may be involved. The mode of action of the leucocyte emigration factor is of course unknown but in view of the known enzymic properties of leucocytes it seems possible that the active principle of leucocyte extracts may be a mucopolysaccharidase or other enzyme which attacks the constituent substances of the vessel wall. When large numbers of leucocytes have collected in the tissues, pus may be formed. Pus is the creamy viscous fluid derived from the autolysis of dead leucocytes (although it may contain the liquefied remains of other tissue cells in addition). It is probably formed by the action of proteases liberated from the dead leucocytes on the proteins of these and other cells.

The process of repair whereby acutely inflamed tissues are restored to normal or converted to an avascular scar is complex. Polymorphs are replaced by macrophages, lymphocytes and sometimes plasma cells, fibroblasts migrate into the region and lay down reticulum and collagen and new blood vessels, lymphatics and nerves also grow into the damaged area. It seems very likely that these events are controlled by locally released or activated endogenous substances. Unfortunately almost nothing is known of these hypothetical local hormones. The cortisone group of drugs is very effective in inhibiting the process of repair and it may be that their action will one day provide a clue as to the nature of the controlling mechanism.

The actual process of collagen formation is thought to begin as the elaboration of mucopolysaccharide ground substance by fibroblasts and macrophages and possibly by vascular endothelium. This then appears to be followed by secretion of a protein termed procollagen from macrophages and fibroblasts. Pro-collagen has a molecule of globular configuration which is converted to the fibrillar molecule of collagen possibly by interaction with ground substance. In vitamin C deficiency, in which wound healing is notoriously slow, ground substance is formed normally but the later stages fail to develop. Cortisone on the other hand acts at an earlier stage by inhibiting migration and proliferation of fibroblasts. It has been suggested that this is in fact secondary to similar inhibition of new blood vessel formation and migration.

PAIN

Some of the pain felt in inflamed tissues is undoubtedly due to direct stimulation of sensory nerve endings. The accumulation of fluid exudate in a confined tissue space would itself cause such stimulation and lead to prolonged pain. On the other hand, many endogenous substances released or activated by injury cause pain on injection or application to a raw surface such as a blister base. Thus histamine is moderately active in this respect, as is acetyl choline. 5-H.T. is a much more powerful pain-producer and so are a variety of peptides of the kinin type. The globulins themselves do not cause striking pain on injection nor, as far as is known, do the substances that cause leucocyte emigration. In another category the nucleoside xanthosine and its base xanthine cause intense pain on injection in very small amounts (Moulton *et al.*, 1954). Thus there are many suitable endogenous substances whose liberation might account for pain in inflammation. It is not yet possible to say which are the more important at any particular stage in the inflammatory cycle. However, it is known that release of 5-H.T. occurs very soon after injury. In addition, bradykinin, a pain-producing peptide, is formed in plasma within a minute or two of the fluid making contact with glass. These two endogenous compounds are therefore at the moment the most likely candidates for the role of chemical mediator of pain in inflammation (Armstrong *et al.*, 1957).

FEVER

It might be thought that fever which is so much a feature of infections of all kinds is a direct result of the invading organisms on the body. However, pyrexia occurs also in disease not associated with invasion by microorganisms, particularly if there has been destruction of tissue, e.g. in burns or infarction (death of tissue due to sudden loss of blood supply as in the heart in coronary thrombosis).

It has been known since 1884, that pus will cause fever if injected into animals. This effect was later attributed to bacterial products when it was discovered that endotoxin, i.e. extracts of dead bacteria, especially gram-negative bacilli, also caused pyrexia on injection. These endotoxins are polysaccharides or lipopolysaccharides and being relatively heat-stable are apt to contaminate even sterilised solutions and apparatus, constituting the so-called pyrogens. Similar pyrogenic polysaccharides are present in many animal and plant tissues (Bennett and Cluff, 1957).

When endotoxin or similar polysaccharides are injected, the resultant fever is characteristic. There is a latent period before the body temperature begins to rise and in addition the number of circulating white cells falls (leucopenia) followed by a rise (leucocytosis). Furthermore, repeated injections of endotoxin lead to a state of tolerance in which further injections fail to cause pyrexia. Following repeated doses of one endotoxin, tolerance develops to endotoxins from other bacterial species and also to the pyrogenic action of infections and trauma. This refractory state lasts for about two weeks and is not an immunological phenomenon.

Since these polysaccharide pyrogens are present in both bacteria and tissues they might in theory account for fever in both infective and non-infective states. The falsity of this assumption was demonstrated by the extraction from polymorphonuclear leucocytes of another quite different substance that also caused fever on injection. This leucocytic pyrogen, as it is called, is followed on injection by fever, with the intervention of only a very short latent period. In addition, repeated injections do not call forth tolerance and animals tolerant to endotoxin respond to leucocyte extracts with fever. Finally, leucocytic pyrogen does not cause significant leucopenia.

The relationship between endotoxin and leucocytic pyrogen was then studied. It was found that injected endotoxin disappeared rapidly from the blood stream and that simultaneously, the level of another circulating pyrogen with the properties of leucocytic extracts rose. The concentration of this endogenous pyrogen was related to the height of pyrexia. As a result of this experiment it was suggested that endotoxin acts by damaging leucocytes which then liberate endogenous pyrogen. Endotoxin is known to cause leucocytes to adhere to vessel walls and this might be associated with release of their pyrogen. It now seems more likely, however, that endotoxin acts directly on the leucocytes. Endogenous pyrogen indistinguishable from leucocytic pyrogen has been demonstrated in the blood of rabbits at the height of fever due to influenza virus, pneumococcal septicaemia and non-septicaemic streptococcal cellulitis.

At this stage it seemed probable that fever was due largely to liberation of endogenous pyrogen, derived probably from polymorph leucocytes. This view was confirmed by experiments showing that endogenous pyrogen caused fever more quickly if it was injected directly into the arteries supplying the thermo-regulatory centre than if given into a peripheral vein. Endotoxin caused an equally delayed pyrexia given by both routes.

However, later work showed that the initial phase of fever following injection of endotoxin is probably due to a direct action of the endotoxin and that it is only after some hours have elapsed that fever can be said to be wholly due to endogenous pyrogen. Furthermore, it has also been demonstrated that an endogenous pyrogen indistinguishable from that of leucocyte extracts is present in sterile exudates even in the virtual absence of leucocytes brought about by administration of nitrogen mustard (Bennett and Beeson, 1953). Little is known of the chemical nature of endogenous pyrogen. It is a non-diffusible and therefore large molecule and relatively heat-labile. A likely guess would be that it is a glycoprotein or mucoprotein.

It seems, therefore, that at least four pyrogenic compounds may be operative in injury and inflammation; polysaccharides derived from microorganisms or from damaged tissues and endogenous pyrogen derived from polymorph leucocytes and at least one other source. Many tissues have been studied but so far only polymorph leucocytes have yielded an extract with the properties of endogenous pyrogen (Wood, 1958).

LEUCOCYTOSIS

No aspect of the phenomena associated with inflammation has proved more intractable than leucocytosis. This is due partly to the complexity of the response itself. Thus in inflammation due to pyogenic microorganisms or certain viruses, e.g. poliomyelitis and rabies, or associated with sterile but extensive destruction of tissue, there is an increase in the level of circulating polymorph leucocytes. However, other infections, bacterial and viral are associated with a drop in the number of circulating polymorphs. In this category are typhoid fever, brucellosis, influenza and measles. Moreover, in other virus diseases, notably glandular fever and pertussis, there is an increase in the level of circulating lymphocytes. In other conditions, usually of an allergic or parasitic origin there is an increase in the number of circulating eosinophil leucocytes.

The situation is complicated further by the fact that, unlike other changes associated with inflammation, transient leucocytosis occurs frequently as a physiological phenomenon, e.g. after muscular exertion, both voluntary and involuntary, fear, mental stress generally and prolonged mental concentration. Leucocytosis occurs also in pathological situations in which inflammation or tissue destruction are absent, e.g. acute haemorrhage and profound chilling.

These observations suggest that the polymorphonuclear leucocytes detected in the above circumstances may be associated with the concept of "stress." It is suggested that stress leads to release of adrenaline which in turn stimulates the pituitary to release ACTH which in turn stimulates the release of glucocorticoids from the adrenal cortex (Selye, 1950). In fact, adrenaline, ACTH and cortisone all cause a polymorph leucocytosis if injected into suitable animals. Hypothalamic stimulation in one of a pair of parabiotic rabbits leads to a polymorph leucocytosis in both animals, the response being abolished by section of the splanchnic nerves (Rosenow, 1951). This result is consistent with neurogenic stimulation of the adreno-pituitary system but also with the release of marrow stimulating substances from other organs, e.g. liver (Beer, 1948) and also with direct neurogenic activation of the bone marrow.

In the polymorph leucocytosis associated with inflammation similar mechanisms may be operative. It seems unlikely, however, that other factors fail to play a part, especially in view of the sustained nature of the leucocytosis seen, e.g. in some infections.

The polysaccharides and lipopolysaccharides known as bacterial endotoxins cause leucocytosis (preceded by leucopenia) on injection into rabbits. It is possible that bacterial protein, too, may be involved since this material also causes neutrophil leucocytosis on injection. The effect may be indirect and mediated through the nervous system since narcotics have been claimed to abolish this effect. Milk is probably more effective than bacteria as a leucocytosis-promoting factor, so that foreign substances in wide variety may share this property. Tissue extracts in general have not been found to cause striking leucocytosis although nucleic acids possess some ability to do so. Extracts of leucocytes with powerful pyrogenic properties (see above) do not cause significant leucocytosis. Menkin has found certain fractions of inflammatory exudates to cause neutrophil leucocytosis and others to cause neutropenia. He believed these to be globulins or peptides but they have not been fully characterised and it is possible that the active principle was partly bacterial or tissue polysaccharide.

Thus the chemical basis of polymorph leucocytosis following tissue injury remains very obscure. About the more specialised reactions of white cells, such as eosinophilia and lymphocytosis, even less is known.

Chapter 12

SHOCK AND RELATED PHENOMENA

THE GENERAL METABOLIC REACTION TO INJURY

UP TO THIS POINT, injury has been considered in terms of events within the cell or in the immediate vicinity of areas of damage. The problem of injury, particularly trauma, can however also be considered in terms of the general metabolic reaction of the body. This general reaction is intimately related to the question of "stress." The concept of a "stress reaction" is not new but has been developed particularly by Selye into a general hypothesis whose basis is the stimulation of the secretion of the adrenal cortex by injury of many types. The essential feature of the stress hypothesis is that many of the phenomena of the general reaction to injury are due to the effects of the adrenal cortical hormones thus released into the circulation.

The general reaction to injury may be divided into the immediate phase, the phase of shock and the phase of recovery. The most striking feature of the immediate phase is an alteration of carbohydrate metabolism in the direction of increased utilisation. Thus in this stage of injury (the first twelve hours or so) there is an elevated level of blood glucose, a decline in the level of glycogen in skeletal muscle and a rise in the level of pyruvate and lactate in the blood without alteration in their ratio one to the other. There is also a transient increase in the glycogen content of liver. If the body, particularly the liver, is depleted of glycogen prior to injury the rise in blood glucose concentration is considerably lessened.

The key to these reactions is adrenaline. After injury they do not occur in the absence of the adrenal medulla, the chief source of bodily adrenaline. In addition, the changes listed above can be reproduced in a normal animal by injection of adrenaline (Cori, 1931). The secretion of adrenaline in response to stress is of course a cardinal feature of the reactions to danger and other stressful situations as described some time ago by Cannon (1923) and others.

Although release of adrenaline from the suprarenal medulla is a vital feature of the immediate response to injury, its action on carbohydrate metabolism in this situation appears to be partly indirect. Thus in the absence of the pituitary gland the characteristic adrenaline effects on blood sugar are greatly diminished. In addition, the action of adrenaline on carbohydrate metabolism may be largely reproduced by injections of adrenocorticotropic hormone (ACTH) from the pituitary. These observations indicate that some of the actions of adrenaline in the immediate reaction to injury are due to increased secretion of ACTH which in turn causes increased secretion of cortical hormones from the suprarenal. In fact adrenaline causes increased adrenocortical secretion (Vogt, 1943) and depletes the ascorbic acid and cholesterol content of the adrenal cortex (Long and Fry, 1945), a sign of increased secretion. ACTH has similar effects. There is some evidence that the alterations in carbohydrate metabolism depend on the integrity of the hypothalamus (De Groot and Harris, 1950) and it is known that secretion of ACTH may be induced directly by nervous stimuli (Vogt, 1952). Thus it may be that adrenalin stimulates the pituitary by way of the hypothalamus.

Injury__Adrenal__Adrenaline__Hypothalamus__
medulla
Ant. Pituitary__ACTH__Adrenocortical hormones

Apart from the changes in blood glucose, etc., already described, there are at least five common sequelae to injury, indications of increased adrenocortical function, all of which may be reproduced by injection of ACTH and none of which occur in the absence of the adrenal glands. They are a manifold increase in the excretion of steroids derived from the adrenal cortex (Venning *et. al.*, 1944), enlargement of and a decreased level of ascorbic acid and cholesterol in the adrenal cortex (Tefferman *et al.*, 1943), shrinkage of lymphoid tissue in the body and a decrease in the level of circulating eosinophil leucocytes and lymphocytes (Selye, 1946).

The second stage in the metabolic reaction to injury is the phase of shock. Shock itself will be considered later. The chief metabolic features of this period (whose relationship to the circulatory changes of shock are obscure) are an increased level of blood amino nitrogen due either to decreased deamination in the liver (possibly due to hepatic anoxia) or to loss of amino acids from muscle (Engel *et al.,* 1944) and a general blockage of carbohydrate breakdown (Threlfall and Stoner, 1954) . This last disturbance is manifest as a reduced output of energy leading to lowered body temperature and decreased oxygen consumption and as a general depletion of bodily carbohydrate and breakdown of labile phosphate esters especially creatine phosphate, possibly in response to lack of oxidisable substrate (Ord and Stocken, 1955) . It is thought that this disturbance of carbohydrate metabolism, too, may be due partly to increased secretion of adrenocortical hormones causing reduced glucose utilisation (Engel, 1951) .

The chief metabolic feature of the recovery phase of injury, seen after twenty-four hours or so have elapsed, is the increased excretion of urea nitrogen, sulphur and phosphorus (Cuthbertson, 1954) . Since nitrogen loss varies directly with the protein level of the diet the source of these substances appears to be the more labile and rapidly metabolised proteins of the body particularly the liver and skeletal muscles. There seems to be in fact an abnormally rapid breakdown of such protein in this phase of injury and the abnormal catabolism can be partially prevented by administration of carbohydrates, suggesting that increased glyconeogenesis (formation of glycogen from protein) is responsible. The rapid breakdown of protein and consequent formation of glycogen can be reproduced by injection of ACTH and adrenocortical hormones and, as in injury, can be prevented by administration of carbohydrate (Long *et al.,* 1940) . Moreover, loss of nitrogen after experimental injury does not occur if the animal has been previously adrenalectomised. Thus once again it would appear that the changes observed are due to stimulation of the adrenal cortex. However, it has been shown that injury will in fact cause increased nitrogen excretion in adrenalectomised animals provided that a small maintenance dose of adrenocortical extract is given. It seems, therefore, that although injury cannot cause excessive protein breakdown in the absence of circulating cortical hormones, this metabolic reaction is not due directly to stimulation of the adrenal cortex (Ingle *et al.,* 1947; Engel, 1951; Campbell *et al.,* 1953) . In what fashion injury and adrenocortical secretion interact to increase protein breakdown remains obscure.

THE CHEMISTRY OF SHOCK

Shock is a state of peripheral circulatory collapse whose essential feature is that the volume of circulating blood is too small relative to the volume of the blood vessels. This discrepancy may follow either a rapid reduction in the blood volume or a paralytic dilatation of the peripheral vessels or both. Thus shock may occur as a sequel to acute haemorrhage, severe physical, chemical or thermal injury or overwhelming infection. The clinical picture of shock is characteristic and familiar, the patient being cold, pale, sweating and unconscious. The obvious treatment of shock is to increase the blood volume by transfusion. This measure is usually successful, but sometimes it fails. Shock which fails to respond to blood transfusion is called irreversible and has led to much research and speculation on its nature, which nevertheless remains uncertain. Much work on the haemodynamic disturbance of shock has been done on both sides of the Atlantic, particularly during the last war. Accounts of this aspect of the problem may be found elsewhere (Cameron, 1958) .

Shock is an almost perfect illustration of the truism that pathology is the interaction of injury and bodily reaction to injury. When the volume of blood relative to that of the vessels falls suddenly the body reacts by a profound vasoconstriction in all those organs which are not immediately essential for life, i.e. everywhere except the heart, respiratory muscles and brain. Some ill effects may follow this vasoconstriction alone, e.g. in the kidney where there may be tubular necrosis. Elsewhere, particularly in the liver and intestine, following prolonged vosoconstriction, the vessels may develop a form of paralysis often termed decompensation in which they suffer paralytic dilatation and become unable to respond to vasoconstrictor stimuli. The failure of these vessels to regain their normal tone is one of the essential features of irreversible shock.

The compensatory phenomena of shock, e.g. changes in cardiac output and peripheral vasoconstriction, may be attributed to nervous and hormonal changes (especially concerning the adrenal medulla) of a physiological nature and need not be discussed here. Decompensation of peripheral vessels however is a suitable criterion of shock on which to base an account of its chemistry.

THE ROLE OF VASOCONSTRICTOR AND VASODILATOR AMINES

One of the most popular views of the pathogenesis of vascular decompensation was that which suggested a massive release of the vasodilator histamine. This theory went out of favour for a variety of reasons, including the sustained nature of the circulatory collapse and the failure of antihistamine drugs as a therapeutic measure. Nevertheless, there is no doubt that release of histamine does occur (Bloom, 1944).

It is equally clear that release of the vasoconstrictor amines adrenaline, nor-adrenaline and 5-H.T. also takes place. These vasoconstrictor amines are also implicated in the sense that a cardinal feature of shock is the inability of vessels in a state of decompensation to respond to adrenaline and nor-adrenaline in the normal fashion. The infusion of large doses of adrenaline or nor-adrenaline leads paradoxically to phenomena suggestive of massive histamine release and to a state of peripheral circulatory collapse resembling shock. This latter observation seemed to indicate that decompensation in shock might be due to the development in certain parts of the vascular bed of a refractory state in which the vessels, because of over-stimulation with adrenaline or nor-adrenaline, lost the ability to respond to these vasoconstrictor amines or indeed to any stimulus leading towards a restoration of vascular tone (Zweifach, 1958).

Schayer (1960) has recently shown the way towards a possible reconciliation of the histamine and adrenaline hypothesis in shock by demonstrating that adrenaline causes increased activity of the enzyme histidine decarboxylase and presumably therefore increased production of histamine. This finding suggests that these two amines might form part of a balance governing vascular tone. The results also indicate that a mechanism exists for prolonged histamine effects and for the paradoxical vasodilator actions of large doses of adrenaline.

Further support for the role of amines in shock comes from the observation that prior depletion of bodily stores of histamine, adrenaline and 5-H.T. by repeated injections of the histamine liberator compound 48/80 renders animals resistant to the onset of shock otherwise induced by a variety of means (Zweifach, 1958).

FERRITIN (VDM) AND THE LIVER

It is possible to demonstrate in the blood in the early stages of shock, a substance that sensitises vessels to the action of adrenaline. This substance has been called vaso-excitor material (VEM) but has not been fully characterised nor has its possible role in shock been elucidated. It is believed, however, to originate in the ischaemic kidney.

Another substance with the opposite property of depressing vascular reactivity to adrenaline is present in the blood in the later and deeper stages of shock. This is known as vasodepressor material (VDM) and has been characterised as a derivative of an iron-binding protein, ferritin (Shore *et al.*, 1951). The source of VDM is the liver and its formation may be an indication of the abnormal circulatory condition of the liver in shock. It may be significant that viviperfusion of the liver with arterial blood protects against the onset of shock in traumatised animals (Zweifach, 1958). On the other hand, the presence of VDM in the circulation need not indicate a general effect on the blood vessels of the body since its appearance in the blood may indicate merely an "overflow" from the liver. VDM may be formed from ferritin by reduction to the —SH form, due to accumulation of reduced xanthine oxidase due in turn to increased purine metabolism in the liver (Green *et al.*, 1956). The iron may undergo chelation to form a complex akin to cytochrome *c*, capable of catalysing the oxidation of adrenaline.

BACTERIAL PRODUCTS

In the past decade there has been a revival of interest in the possibility that bacterial products, in particular those absorbed from the intestine, might contribute to the phenomena of shock (Fine *et. al.*, 1952). A great deal of evidence has now been collected bearing on this point. Bacterial endotoxin (obtained chiefly from gram-negative bacilli) whose active principle is mainly polysaccharide or lipopolysaccharide in nature will cause shock in animals if injected in suitable quantities. As in shock proper this injection causes first a heightened, then a lessened responsiveness of the vessels to adrenaline (Thomas, 1956; Zweifach and Thomas, 1957). Unlike shock, however, endotoxin causes hypertonicity of venules. Nevertheless, rats and rabbits rendered tolerant to endotoxin by repeated injection are thereby protected from shock due to haemorrhage or trauma (Zweifach *et al.*, 1957). Moreover, the blood of shocked animals including those suffering from acute haemorrhage where infection is an unlikely complication contains a substance with many of the properties of bacterial endotoxin (Smiddy and Fine, 1958; Schweinberg *et al.*, 1957).

It is known that prolonged hypotension delays the clearance of bacteria from the circulation (Schweinburg *et al.*, 1954). It is also known that in shock, circulation through the intestine is very poor and that devitalisation of the bowel wall is likely to occur, with increased likelihood of the passage of bacteria and bacterial products into the circulation. In fact, in some circumstances, shock may be prevented by perfusing the intestine of injured animals with arterial blood (Lillehei, 1956). Furthermore, not only do shocked animals exhibit reduced levels of endogenous bacteriostatics, e.g. Properdin, (Frank *et al.*, 1955), but they are in addition hypersensitive to the effects of injected endotoxin and bacteria (Schweinburg and Fine, 1955). Moreover, resistance to shock induced by repeated small traumatic stimuli may be overcome by infection. Finally, it was found, and confirmed in many different species and circumstances, that oral antibiotics, including some compounds not absorbed from the bowel, protected against shock due to trauma or haemorrhage (Frank *et al.*, 1952).

However, not all the evidence favours the view that endotoxin is a major factor in shock. Thus although shocked animals are hypersensitive to endotoxin, guinea pigs rendered sensitive to endotoxin by other means are not hypersensitive to shock. Conversely, rats rendered resistant to shock by injection of yeast extract or denatured albumin are not resistant to endotoxin (Zweifach, 1958).

Finally, it has been found that rats reared to be completely germ-free nevertheless develop shock in the usual fashion when suitably injured (Zweifach *et. al.,* 1958).

TOXIC TISSUE PRODUCTS

The appearance of shock in germ-free rats coupled with the evidence of the importance of endotoxin in shock suggests that damaged tissues themselves might liberate substances with endotoxin-like properties. In fact, polysaccharides of this type are known to exist in a wide variety of animal and plant tissues and may well contribute to the phenomena of shock (Lardy and Shear, 1957). A great deal of work has been done in injecting tissue fluid and extracts from damaged parts of the body into animals and observing shock-like effects. Few of these active principles have been adequately characterised. Proteins and peptides may contribute to their effects but tissue polysaccharides of the endotoxin type may also be involved. Proteolytic enzymes may be involved in the development of shock. Thus shock increases the activity of the proteases in blood whereas animals rendered resistant to shock maintain a normal level of blood protease activity after injury. Proteolytic activity may be linked in some way with the action of endotoxin since injected endotoxin increases the proteolytic activity of blood and since the presence of increased proteolytic activity is associated with hypersensitivity to the actions of endotoxin.

THE RETICULO-ENDOTHELIAL SYSTEM

One of the more curious features of experimental shock is the evidence recently reviewed (Zweifach, 1958) implicating the phagocytic properties of the cells of the reticulo-endothelial system (RES). These cells line sinusoids in liver (Kupffer cells), spleen, lymph nodes and elsewhere.

Thus animals rendered resistant to shock by treatment with antibiotics, or autonomic-blocking drugs or repeated small traumata lose their resistance if the RES is saturated and blockaded by injection of thorotrast or carbon. Conversely, both antibiotics and autonomic-blocking drugs will reverse the depression of phagocytic activity in the RES induced by injection of endotoxin.

Furthermore, animals the phagocytic properties of whose RES has been diminished by blockage show increased sensitivity to both shock and endotoxin (Beeson, 1947). Similarly, rats and rabbits suffering from haemorrhagic shock exhibit striking depression of the phagocytic clearance properties of the RES (Zweifach and Benacerraf, 1958). On the other hand, stimulation of these activities of the RES by injections of non-toxic colloids induces resistance to shock in rats. In addition, in shocked animals the RES cannot be stimulated in this fashion and RES blockade induced experimentally in shocked animals lasts for three to five days as opposed to eight to twelve hours in normal animals.

The precise role of the RES in shock remains uncertain. It is known, however, that RES blockade induces hypersensitivity of

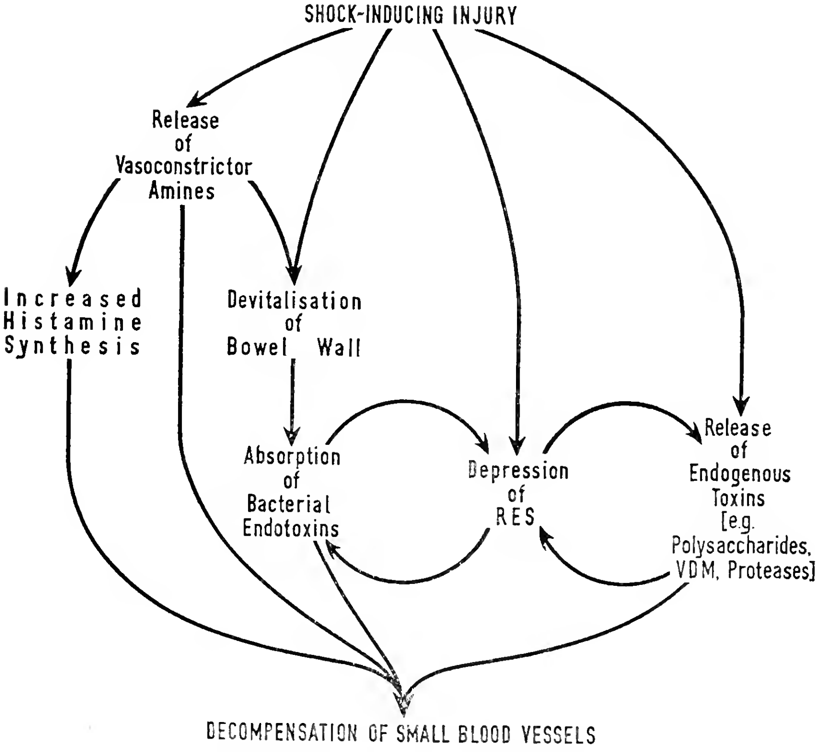

SHOCK-INDUCING INJURY
Release of Vasoconstrictor Amines
Increased Histamine Synthesis
Devitalisation of Bowel Wall
Absorption of Bacterial Endotoxins
Depression of RES
Release of Endogenous Toxins [e.g. Polysaccharides, VDM, Proteases]
DECOMPENSATION OF SMALL BLOOD VESSELS

Fig. 11. Some Suggested Factors in the Mechanism of Shock.

peripheral vessels to the actions of endotoxin. It seems quite likely, therefore, that the role of these phagocytic cells is to remove endotoxin from the circulation. It is possible, too, that the RES is necessary for the inactivation of other endogenous shock-producing substances. Thus there is evidence that inactivation of VDM depends upon the workings of the RES. The liver may be implicated here since in shocked animals, uptake of carbon particles by the Kupffer cells of the RES is confined to the periportal areas instead of being uniform throughout the lobule as in normal animals (Zweifach and Benacerraf, 1958). The formation of toxic compounds may be also checked by the RES, since blockade of this system is said to increase proteolytic activity in blood.

SUMMARY

Figure 11 depicts the possible inter-relationship of some of the changes described in the preceding sections that may lead to decompensation of parts of the peripheral circulation during shock. It is not intended to imply, however, that all the phenomena and profound metabolic disturbances of irreversible shock are due to vascular decompensation alone.

REFERENCES

Alksne, J. F. (1959) : *Quart. J. exp. Physiol., 44*:51.

Armstrong, D., Jepson, J. B., Keele, C. A. and Stewart, J. W. (1957) : *J. Physiol., 135*:350.

Becker, E. L., Austen, K. F. and Marcus, D. M. (1959) : *Brit. J. exp. Path., 40*:494.

Beeson, P. B. (1947) : *Proc. Soc. exp. Biol., 64*:146.

Beer, A. G. (1948) : *Med. Klin., 43*:409.

Bennett, Jr., I. L. and Beeson, P. B. (1953) : *J. exp. Med., 98*:477, 493.

Bennett, Jr., I. L. and Cluff, L. E. (1957) : *Pharmacol. Rev., 9*:427.

Cameron, G. R. (1958) : In *General Pathology*. Editor, H. W. Florey, 2nd ed. London. Lloyd-Luke. p. 206.

Campbell, R. N., Sharp, G. M. E., Boyne, A. W. and Cuthbertson, D. P. (1953) : *Nature, 172*:158.

Cannon, W. B. (1923) : *Traumatic Shock*. New York, Appleton-Century.

Cuthbertson, D. P. (1954) : *Brit. Med. Bull., 10*:33.

Cori, C. F. (1931) : *Physiol. Rev., 11*:143.

DeGroot, J. and Harris, G. W. (1950) : *J. Physiol., 111*:335.

Engel, C. F. (1951) : *Amer. J. Med., 10*:556.

Engel, F. L., Harrison, H. C. and Long, C. N. H. (1944) : *J. exp. Med., 79*:9.

Fine, J., Frank, H. A., Schweinburg, F. B., Jacob, S. W. and Gordon, T. (1952) : *Ann. N. Y. Acad. Sci., 55*:429.

Frank, E., Fine, J. and Pillemer, L. (1955) : *Proc. Soc. exp. Biol., 89*:223.

Frank, H. A., Jacob, S. W., Schweinburg, F. B., Goddard, J. and Fine, J. (1952) : *Amer. J. Physiol., 168*:430.

Glenner, C. G. and Cohen, L. A. (1960) : *Nature, 185*:846.

Goodfriend, T. (1958) : *L'Auto-Intoxication apres Brulure.* Editon Arcia. Brussels.

Green, S., Mazur, A. and Shorr, E. (1956) : *J. biol. Chem., 220*:237.

Harris, H. (1954) : *Physiol. Rev., 34*:529.

Hilton, S. M. and Lewis, G. P. (1955) : *J. Physiol., 129*:253.

Ingle, D. J., Ward, E. O. and Kuizenga, M. H. (1947) : *Amer. J. Physiol., 149*:510.

Landy, M. and Shear, M. J. (1957) : *J. exp. Med., 106*:77.

Lewis, G. P. (1959) : *J. Physiol., 147*:458.

Lillehei, R. C. (1956) : *Amer. J. Physiol., 187*:614.

Long, C. N. H. and Fry, E. G. (1945) : *Proc. Soc. exp. Biol., 59*:67.

Long, C. N. H., Katzin, B. and Fry, E. G. (1940) : *Endocrinol., 26*:309.

Loola, K. D., Calle, J. D. and Schachter, M. (1960) : *J. Physiol., 152*:75.

McCluskey, R. T. and Benacerraf, B. (1959) : *Amer. J. Path., 35*:275.

Meier, R. and Schär, B. (1957) : *Hoppe-Seyl. Z., 307*:103.

Menkin, V. (1956) : *Biochemical Mechanisms in Inflammation,* 2nd ed. Thomas, Springfield.

Miles, A. A. and Wilhelm, D. L. (1955) : *Brit. J. exp. Path., 36*:71.

Moon, V. H. (1944) : *Brit. med. J., 1*:773.

Moulton, R., Spector, W. G. and Willoughby, D. A. (1957) : *Brit. J. Pharmacol., 12*:365.

Ord, M. G. and Stocken, L. A. (1955) : *Biochem. J., 59*:272.

Rosenow, G. (1951) : *Acta haematol. (Basel), 5*:1.

Rowley, D. A. and Benditt, E. P. (1956) : *J. exp. Med., 103*:399.

Schayer, R. W. (1960) : *Science, 131*:226.

Schweinburg, F. B., Shapiro, P. B., Frank, E. D. and Fine, J. (1957): *Proc. Soc. exp. Biol., 95*:646.

Schweinburg, F. B., Frank, H. A. and Fine, J. (1954) : *Amer. J. Physiol., 179*:532.

Schweinburg, F. B. and Fine, J. (1955) : *Proc. Soc. exp. Biol., 88*:589.

Selye, H. (1946) : *J. clin. endocrinol., 6:*117.

Selye, H. (1950) : *Stress.* Montreal, Acta Inc.

Setnikar, I., Salvaterra, M. and Ternelcou, O. (1959) : *Brit. J. Pharmacol., 14:*484.

Shorr, E., Zweifach, B. W., Furchgott, R. F. and Baez, S. (1951) : *Circulation, 3:*42.

Smiddy, F. G. and Fine, J. (1958) : *Proc. Soc. exp. Biol., 96:*558.

Smith, M. J. H. (1959) : *Annals Rheum. Dis., 18:*298.

Spector, W. G. (1958) : *Pharmacol. Rev., 10:*475.

Spector, W. G. and Willoughby, D. A. (1959) : *J. Path. Bact., 78:*121.

Spector, W. G. and Willoughby, D. A. (1960a) : *J. Path. Bact., 79:*21.

Spector, W. G. and Willoughby, D. A. (1960b) : *J. Path. Bact., 80:*

Spector, W. G. and Storey, E. (1958) : *J. Path. Bact., 75:*383.

Tefferman, J., Engel, F. L. and Long, C. N. H. (1943) : *Endocrinol., 32:* 373.

Thomas, L. (1956) : *J. exp. Med., 104:*865.

Threlfall, C. J. and Stoner, H. B. (1954) : *Quart. J. exp. Physiol., 39:*1.

Uvnäs, B. (1958) : *J. Pharm. Lond., 10:*1.

Venning, E. H., Hoffmann, M. M. and Browne, J. S. L. (1944) : *Endocrinol., 35:*49.

Vogt, M. (1952) : *J. Physiol., 118:*588.

Vogt, M. (1943) : *J. Physiol., 102:*341.

Wilhelm, D. L. (1959) : *J. Physiol., 148:*9P.

Wilhelm, D. L., Mill, P. J., Sparrow, E. M., Mackay, M. E. and Miles, A. A. (1958) : *Brit. J. exp. Path., 39:*228.

Wolstenholme, G. E. W. and O'Connor, C. M., Edits. (1956) : Ciba Foundation Symposium on Histamine. Churchill, London.

Wood, B. W., Jr. (1958) : *New Eng. J. Med., 258:*1023.

Zweifach, B. W. (1958) : *Brit. J. Anaesth., 30:*486.

Zweifach, B. W. and Benacerraf, B. (1958) : *Circulat. Res., 6:*83.

Zweifach, B. W., Benacerraf, B. and Thomas, L. (1957) : *J. exp. Med., 106:*403.

Zweifach, B. W., Gordon, H. A., Wagner, M. and Reyniers, J. A. (1958): *J. exp. Med., 107:*437.

Zweifach, B. W. and Thomas, L. (1957) : *J. exp. Med., 106:*385.

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